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On the Descending Spinal Pathways Subserving the Sweating of the Plantar Sweat Glands in Rats

By

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It is not long since the evidences of secretion in the plantar sweat glands of rats were given.

Ring and Randall[1] were the first to study histologically the plantar sweat glands in rats. Further, they found that the sweating was causable by the prolonged continuous stimulation of the sciatic nerve of the corresponding side. They failed, however, to induce sweating by raising the body temperature.

Nakagawa[2] reported that in the plantar surface of non-anesthetized albino rats a spontaneous sweating was always observed, which was completely abolished by ether anesthesia or by the section of the sciatic nerve on the same side.

It is the purpose of the present investigation to elucidate the spinal pathways subserving this spontaneous sweating of the plantar sweat glands in rats.

Experimental

Methods

Adult albino rats, regardless of sex, were experimented on. Under ether anesthesia the lesion of the spinal cord was made with the tip of a needle or of a knife.

In the first series of experiments, the total section of the spinal cord was made at various levels of the thoracic or lumbar segments. In the second series, the hemisection was performed at the thoracic or lumbar level. And, in the third series, the spinal cord was sectioned on one side partially at the thoracic level, the extent of lesion being verified after finishing the experiments by microscopic examination.

The animals were allowed to survive after operation for several weeks and during this period the observations on sweating were made almost every day.

The method used in the present experiments for detecting the sweating was that deviced by Wada and Takagaki[3]; that is, the area of the plantar surface to be examined was painted with an iodine alcohol solution and after drying completely the area was painted with a mixture of starch powder and castor oil. Sweating was evidenced by the appearance of black dots at the openings of the sweat ducts.

Before experiments the spontaneous sweating at the plantar surface of each rat was examined and only those rats were chosen, whose sweating on the left and right hind feet was almost equal in degree.

Results

(1) *Total section of spinal cord at thoracic or lumbar level*

In 9 animals, the total section of the thoracic segment was made at the levels varying from D_1 to D_9. In one of them, whose spinal cord was transected at the level of D_6, the spontaneous sweating in a small degree was observed in the plantar surface of both hind feet. In other 2 rats, which died on the 4th and the 5th day after the total section of the spinal cord at D_1, respectively, a faint sweating was found after section in both plantar surface. In 6 others with totally transected spinal cord at the level of D_3-D_9, spontaneous sweating in both plantar surfaces was completely abolished.

In 2 animals the total section of the spinal cord was made at the level of D_{10}. In one of them sweating was scarcely observed on both plantar surfaces and in the 2nd rat a faint sweating was found.

In 4 animals the total sections were made at the levels varying from D_{11} to L_1. In them, it was noticed that the more caudal was the level of the total section the more profuse was the sweating.

In 2 rats the section was performed at the level of L_2 and L_3, respectively. In these cases, no effect of section was observed. Further, a profuse sweating was observed in another rat, whose spinal cord was destroyed below the level of L_2.

From these experiments it may be concluded that centres for the spontaneous sweating of the plantar surface in rats are situated at somewhere above the thoracic level and that the secretory fibres descend the thoracic segments and leave the spinal cord at levels of D_{11}-L_1.

(2) *Hemisection of spinal cord at thoracic or lumbar level*

In 8 animals the left lateral half of the spinal cord was sectioned at the levels varying from D_1 to D_9.

There, spontaneous sweating of the ipsilateral plantar surface was found to be significantly reduced.

In 5 rats the left hemisection of the spinal cord was made at the levels varying from D_{10} to L_1. In them, the more caudal was the hemisection

level the more insignificant was the difference between the right and left hind feet in the intensity of the plantar sweating.

In 2 others the left hemisection of the spinal cord was performed at the level of L_2 or L_3. No change was observed in these cases.

The experimental results above mentioned show that the secretory fibres for the plantar sweat glands descend the spinal cord and run predominantly without crossing to the ipsilateral hind foot. And the partial decussation of secretory fibres located close to the level of preganglionic outflow is also indicated by a slight spontaneous sweating remaining after the hemisection in the ipsilateral plantar surface.

(3) *Partial lesion of the spinal cord at the thoracic level*

Unilateral section of the ventral quadrant at the level of D_8 was made in 2 rats. Spontaneous sweating in the ipsilateral plantar surface was in these rats obviously reduced. The effect of unilateral section of the dorsal quadrant which was made at D_5-D_8 in 5 rats, resembled that of the ventral quadrant section.

In 4 rats, the lesion was made on the left lateral column at the level of D_7-D_8. In them, spontaneous sweating of the ipsilateral plantar surface was markedly reduced, except for one rat in which the lesion was restricted within the outer portion of the lateral column. Further, it was found that even by the section of the ventral portion of lateral column sponteneous sweating of the plantar surface of the ipsilateral side was definitely reduced.

On the other hand, it was observed in 2 rats that the section of ventral or dorsal column produced no effect on spontaneous sweating in the plantar surface.

From these experimental results it may reasonably be assumed that the pathways of the secretory fibres to the plantar sweat glands are located in the lateral or ventro-lateral column.

Discussion

Descending spinal pathways to the plantar sweat glands in cats were traced by Ott.[4] In the cats, in which various columns of the spinal cord were damaged at the level between D_6 and D_7, spontaneous sweating was induced by the faradization of the medulla. After the hemisection of the spinal cord, sweating was elicited only in the plantar sweat glands of the contralateral foot. The unilateral section of the lateral column abolished the sweating of the ipsilateral plantar surface, whereas no effect was observed of the section of the posterior column. From these experimental results he inferred that the secretory fibres descended the lateral column of cord without crossing.

Quite different evidences were given by Beaton and Leininger[5] as

to the spinal pathways for sweating in monkeys. By the unilateral section of the lateral or ventrolateral column of the spinal cord at the midthoracic level, sweating in the contralateral foot was found to be wholly impaired. Thereby they concluded that descending spinal pathways for sweating of the lower extremity in monkeys were located in the lateral or ventrolateral column, having a complete or almost complete decussation below the midthoracic level.

In human beings it was proved by Foerster[6] and List and Peet[7] that the unilateral section of the lateral column of the spinal cord at the cervical level caused an ipsilateral hemihypohidrosis, suggesting that most of the secretory fibres descend the lateral column of the spinal cord without crossing.

Thus it has been elucidated by the present investigation that the descending spinal pathways for sweating of the plantar surface in rats are similar in their course to those in cats and in human beings and are quite different from those in monkeys.

It may be added here that spontaneous sweating of the plantar sweat gland is also observable in mice. Therefore, it will be of interest to study in mice the spinal descending pathways for sweating.

Summary

The present investigation has been undertaken to elucidate the spinal pathways for spontaneous sweating of plantar sweat glands in albino rats.

Total section, hemisection or unilateral partial section of the spinal cord were made at various levels and the effects of these sections on spontaneous sweating in the plantar surface were examined.

Experiments of the total section of the spinal cord indicated that secretory fibres for the plantar sweat glands in rats descended the thoracic segments of the spinal cord, leaving the spinal cord at the levels from D_{11} to L_1.

Experiments involving the hemisection or partial section of the spinal cord indicated that the descending spinal pathways subserving spontaneous sweating in the plantar surface ran in the lateral or ventrolateral column and mostly without crossing.

I wish to express my gratitude to Prof. T. Suzuki, who suggested this problem to me, for his encouragement and advice.

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The Correlation between Cancer of Uterus and Cancer of Penis

By

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There are some reports[1),2),3)] that seem to suggest a possible correlation between the pathogeneses of the cancer of uterine cervix and the cancer of penis.

The authors of this paper have made researches for such correlations, by way of calculating the crude death rates for cancer of the uterus and for cancer of the penis by the prefectures in Japan, basing ourselves on the vital statistics. Strictly speaking, we should have taken only cancer cases of the uterine cervix into account, but since in Japan, the death certificates rarely bear the distinction between the uterine cervical and the uterine corpus cancers explicitly stated and since more than 95% of the cases of uterine cancer are estimated to be of cervical cancer, we used the figures for uterus cancer for approximate calculation of the death rates for cervical cancer. Seeing that the age distribution is not entirely uniform by prefectures, we should have computed the age-adjusted death rates for all the prefectures, but owing to the lack of adequate data, we had to rely on the crude death rates. The death rates used concern the three years of 1947 through 1949. We have forty-six prefectures in Japan. The total average of crude death rates for the cancer of the uterus for entire Japan stands at 20.2 per 100,000 female population. In the prefecture with the highest rate it is 26.0, and in that showing the lowest figures it is 15.1 per 100,000 women. As for the death rates for cancer of the penis, the nationwide average is 0.15 per 100,000 male population, while by prefectures, the highest rate amounted to 0.45 and the lowest 0. The correlation coefficient between these two series of death rates was found to be +0.335. Upon testing the significance of this coefficient with reference to the table given the Values of the Correlation Coefficient for Different Levels of Significance[4)], we found that it is stochastically significant, at the level of significance not higher than 5%.

From the above results, it may be premature to decide that there is some common factor in the pathogeneses of cancer of the uterine cervix and of the penis, but they may serve as a datum for reference in making inferences on the possible correlation between the two kinds of cancer.

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Crystallographic Studies on the Eggs of Various Human Parasites

I. Observation with Polarization Microscope

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Introduction

In Japan, contamination of the biliary passage by ascaris lumbricoides and other parasites is frequently observed.[1)-4)] According to the investigation of Yasuda[5)] of our clinic, eggs of parasites and cuticule of ascaris were found in high percentage in the central parts of biliary pigment stones, which could not be of judging parasitic origin from the external appearance. Furthermore, we[6)7)] could clinically observe the actual process of precipitation of calicium bilirubinate on eggs of the intestinal parasites. Atsumi,[8)] my colleague in the clinic, also succeeded in precipitating calcium bilirubinate on the surface of ascaris eggs in his animal experiments. These facts show not only that the contamination of the biliary duct by parasites causes change in the composition of bile, but also that the eggs and the cuticule of ascaris stranded in the biliary duct have an important meaning as the nuclei of biliary stones.

The above consideration has led us, members of the clinic, to the carrying on the investigations of the parts that some parasites and their eggs play in offering nuclei for pigment chalk stone formation. As a link in the chain of these studies, I have conducted a crystallographic study of the eggs of various parasites, starting from the investigations on ascaris eggs. This study has led me to some informations on the crystallographic properties and at the same time on the values as nuclei of biliary stones

* Read before the 22nd General Meeting of the Japanese Society of Parasitology, Fukuoka, April 5, 1953.

of the eggs of various parasites, which I will report in the following.

Experimental

Materials and Methods

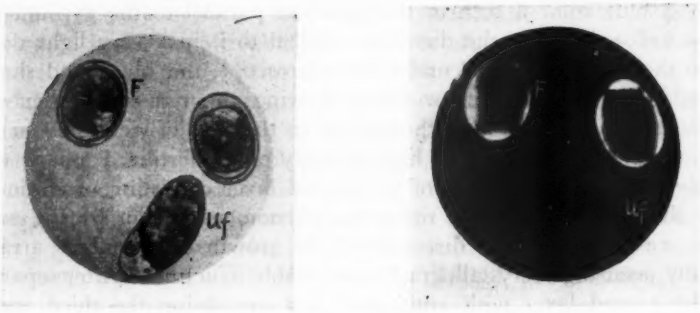
As materials, eggs of ascaris lumbricoides, hookworm, trichostrongylus orientalis, trichocephalus trichiurus and clonorchis sinensis were used and the shells of these eggs were examined in the main under a polarization microscope, as described below. In the case of ascaris eggs, the nature of their albuminoid membranes has also been investigated. The attached illustrations show pictures always in the same magnification of x320 taken with a horizontal-vertical camera of Karl-Zeiss 20 cm in length, using a 500 W incandescent lamp as light source.

Results

1) Eggs of Ascaris lumbricoides

Feritilized eggs. When such an egg is examined with an biological microscope, upon removal of the albuminoid membrane, it is found to be ellipsoid in form about 0.05–0.07 mm. in its major, and about 0.04–0.06 mm. in its minor axes, and covered by a thick and transparent shell which shows a triple outline. The thickness of the shell is rather varied with each egg, ranging between 0.006 and 0.008 mm. approximately. It is known that the shell is formed of a chitinous substance. To ascertain the structure of such a shell, further, examinations were made under a polarization microscope, using a set of crossed nicols prisms under a rotating stage. A fertilized egg of ascaris clearly showed a white interference color band in coincidence with the egg shell, diagonal and unilluminated positions were also located and it was found to show a high degree of double refraction. From this it is clearly inferred that the shell has a crystalline structure. The pictures a and b of Fig. 1 show the photographs of ascaris eggs in the same field of view under a biological and a polarization microscopes illuminated with parallel light rays and light rays passing through crossed nicols plates respectively. As shown in Fig. 1 (F), the comparative thickness, ellipsoid form and crystalline structure of the shell constitute the characteristics of fertilized ascaris eggs that contribute to their high stability.

Now, there are some opinions about the complex structure of the shell of a fertilized ascaris egg. Following Ida's method[9], I separated the shells into individual layers and found out that they consist of four layers, which I will call hereunder the first, second, third and fourth layer respectively counting from the outermost one. The results of the separate examination of each layer showed the following. The first layer, when



a) Biological microscope. b) Polarization microscope (crossed nicols).

Fig. 1. Fertilized and unfertilized eggs of Ascaris lumbricoides
F—Fertilized egg. Uf—Unfertilized egg.

reacted with pure nitric acid, swells up separating itself from the second layer so that the whole major diameter of the egg grows to about 0.11 mm., and the inflated first layer shows scarcely any double refraction. The second layer, however, remains fast and shows high double refraction. When a gypsum detector is used, we find addition and reduction phenomena on the second layer, though not very distinctly, as shown in Fig. 2,

Fig. 2

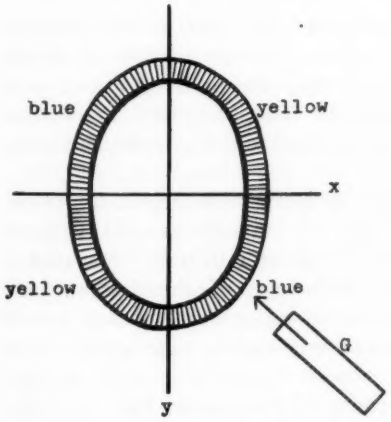


Fig. 3

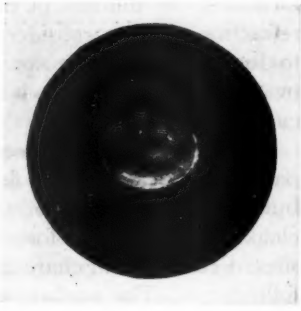


Fig. 2. Schema of the second layer of fertilized Ascaris egg inserting gypsum plate. G: Gypsum plate.

Fig. 3. Ascaris egg upon whose albuminoid membrane deposited experimentally calcium carbonate in the bile. Polarization microscope (crossed nicols).

where a blue color is seen in the direction parallel to the gypsum plate and a yellow color in the direction vertical to it in a very light degree. When the eggs are rotated under the microscope, the blue band showing the addition and the yellow band showing the reduction phenomena remain at the same position throughout in the field of view. From these facts, we may infer that, the high stability of the fertilized ascaris egg is mainly due to the existence of the second layer consisting of chitin and, from the addition and the reduction phenomena shown by the second layer, we learn that the direction of the growth of crystals is arranged radially, assuring a crystallographically stable structure. After separating off the second layer with antiformin and examining the third and the fourth layers, we find they are very thin, are almost completely dark under a polarization microscope and show no double refraction.

Unfertilized eggs. Examining these after removal of the albuminoid membrane, we find they are about 0.08–0.09 mm. in the longer and about 0.04–0.05 mm. in the shorter axes, being larger than the fertilized eggs and more elongated and irregular in form. The shells consist of only two layers and are much thinner than those of the fertilized eggs, the thickness measuring only about 0.003 mm. Under a polarization microscope, the thin egg shells show only a very weak double refraction and are very easily distinguishable from fertilized egg shells. This can be readily seen in the pictures a and b in Fig. 1, (Uf). In b, the unfertilized eggs are nearly unrecognizable.

These characteristics of the unfertilized eggs, that is, the irregular form and the thinness of their shells, and the very low degree of double refraction show a considerable difference from fertilized eggs and seem to indicate that they are easily fractured. The immature unfertilized ova in the ovaries of ascaris have been ascertained to show the same tendency of instability.

The albuminoid membrane. The eggs of ascaris discharged out of the parasite body, whether fertilized or not, are usually covered with albuminoid outer layer of the thickness of about 0.01 mm. No such a cloaking membrane is found in any case of the eggs of other human parasites. The known characteristics of this albuminoid membrane are as follows: 1) The surface shows irregular unevenness, aggregated in irregular granular patches. 2) The membrane is cohesive. 3) It is originally translucent and has the appearance of thin paste, but is readily stainable with bilirubin etc. 4) As may be seen from the fact that it may be easily removed by rubbing the eggs between a pair of slide glasses, it is very easy to separate by external force. 5) It may be readily dissolved in artificial stomach juice and pepsin. I tried to investigate whether, beside the above characteristics, it has the propensity to form a nucleus

of a pigment chalk stone. Observing the membrane with a polarization microscope, it shows no double refraction at all, revealing its amorphous structure. On the other hand when ascaris eggs with albuminoid membrane are kept in a bouillon medium in which bacteria are growing, at a temperature of 38°C, the albuminoid membrane was found to rot, ferment and dissolve away. It is reasonable to assume that ammonium carbonate and similar carbonates will be generated in the course of this process, and if calcium is contained in the medium, the carbonate ions generated in this process would combine with calcium to form crystals of calcium carbonate which will grow by further precipitation. I succeeded in experimentally verifying this hypothesis. 10 cc. of liver bile extracted with a T-tube inserted into the biliary duct after an operation was placed in a test tube, 1 g. of calcium chloride was added to make the calcium content in it about 3.5% and ascaris eggs with their albuminoid membrane were put into it. The mixture was kept at 38°C. The results of microscopic observation one week thereafter showed the eggs with albuminoid membrane were covered with precipitated calcium carbonate, which was seen to have formed beautiful crystals, while the membraneless eggs were free of such precipitation. Fig. 3 shows a picture of such a culture as seen under a polarization microscope. The white patches show grains of precipitated calcium carbonate. Thus, the albuminoid membrane is uneven and sticky on the surface, is easy to stain and ready to receive pigments, and when putrefaction occurs, causes precipitation of calcium compounds, forming a possibly very ideal nucleus of growing biliary pigment stone.

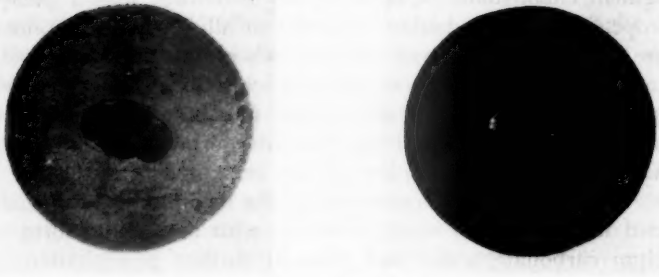
2) Eggs of Ancylostoma duodenale

The fertilized eggs are of elongated ellipsoid in form usually about 0.06 mm. in length and about 0.04 mm. in maximum breadth. Their shells may be divided into two layers, both very thin. When examined under a polarization microscope, they show a very weak double refraction, very slightly observable in coincidence with the shells. This fragile structure of the fertilized eggs of hookworm may be deemed natural, if we consider that these eggs, unlike those of ascaris, are rather early hatched. Fig. 4 a and b show photographs of these eggs under a biological and a polarization microscopes respectively. In comparison with ascaris eggs, they are distinctly different.

3) Eggs of Trichostrongylus orientalis

The fertilized eggs are somewhat larger than hookworm eggs, that is, about 0.08–0.09 mm. in length and about 0.04 mm. in breadth, of elongated ellipsoid in general form, but they are peculiar in their asymmetrical shape.

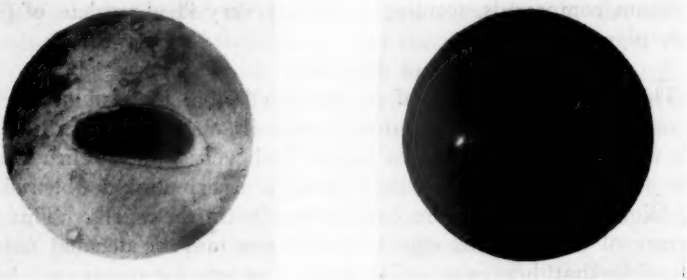
Their shells consist of two layers, thin but thicker if compared with



a) Biological microscope. b) Polarization microscope (crossed nicols).

Fig. 4. Egg of Ancylostoma duodenale.

those of hookworm eggs. When observed under a polarization microscope, the findings are similar to those of hookworm eggs, the shells showing only a low degree of double refraction. When the ends of the ellipsoid eggs are dissimilar in shape, the narrow end has a thicker layer of the shell matter usually, but we found no case where this thick end of the shell showed any particularly high degree of double refraction. The pictures of Fig. 5 a and b show their photographs under a biological and a polarization microscopes respectively, showing an apparent fragility of their shells, like the hookworm eggs.



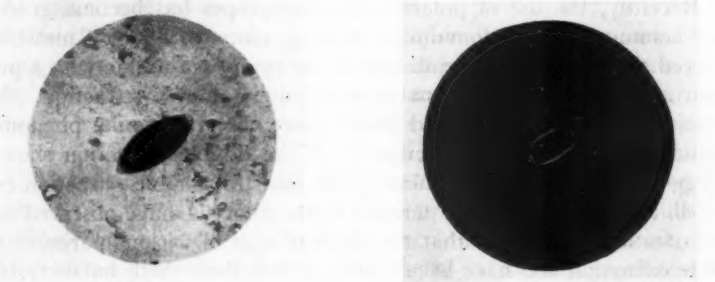
a) Biological microscope. b) Polarization microscope (crossed nicols).

Fig. 5. Egg of Trichostrongylus orientalis.

4) Eggs of Trichocephalus trichiurus

These are about 0.05–0.054 mm. in length and about 0.022–0.033 mm. in breadth, showing a peculiar beer-cask shape. Their shells are rather thick, showing a distinct triple contour. Of the three layers, the outmost is usually stained yellow-brown by feces, but the inner two are transparent. When an egg is subjected to external force, e. g., it is rubbed

between slideglasses, the three layers are ruptured in one crack, never being peeled asunder. This proves that, as described below, the apparently amorphous outmost layer is not readily abrasive as the albuminoid membrane of ascaris eggs, but is rather compactly adhering to the inner layers. When an egg is observed under a polarization microscope, the two inner layers show a comparatively high degree of double refraction, but the outmost layer is utterly dark, showing that it is amorphous. Hitherto, it was assumed that at the stopper-like projections found at both the ends of the eggs, the shell substance is lacking, but when these parts were examined under a polarization microscope, a layer of shell substance somewhat thinner than the two inner shell layers but showing a strong double refraction indicating a crystalline structure was found at each of such projections, for the protection of this part supposed to be of low resisting power. This was a discovery not to be made with a biological microscope. From the above, we may infer that the eggs of this parasite is of high stability. The Fig. 6 a and b show such eggs as seen under a biological and a polarization microscope respectively.

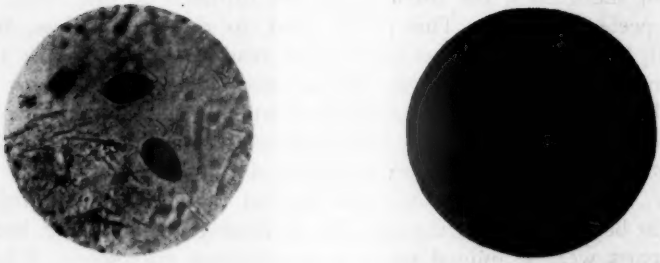


a) Biological microscope. b) Polarization microscope (crossed nicols).

Fig. 6. Egg of Trichocephalus trichiurus.

5) Eggs of Clonorchis sinensis

These are about 0.025–0.033 mm. in length and about 0.015–0.02 mm. in maximum breadth, and show a peculiar pear-like form. Their shells are rather thick, considering the size of the eggs, and are usually stained yellow-brown by bile. When examined under a polarization microscope, a rather high-degreed double refraction appears in coincidence with the shells, clearly showing their crystalline structure. On the helmet-like egg covers, only a thin layer of shell substance showing a low double refraction is found, seemingly indicating that the resistance to external force is lower here than in the other parts of the shell. The pictures a and b of Fig. 7 show some of these eggs as seen under a biological and under a polarization



a) Biological microscope. b) Polarization microscope (crossed nicols).

Fig. 7. Eggs of Clonorchis sinensis.

microscopes respectively. From the above observations, we may conclude that the egg-shells of clonorchis sinensis are of a considerable stability, as are the egg-shells of Trichocephalus trichiurus.

Summary and Discussion

Recently, the use of polarization microscopes has become gradually more common in the domains of biology too. In 1928, Runnström[10] observed the fertilization membrane of the egg of sea-urchin with a polarization microscope and has first pointed out its double refraction. More recently, Schmidt,[11] Inoue and Dan[12] have observed similar phenomena, Schmidt giving a minute description of the double refraction shown in the egg-shells of ascaris and pointing out that this double refraction comes from chitin in the shell. As detailed in the above, I have observed under a polarization microscope that the shells of eggs of various parasites show double refraction and have been informed that these shells have crystalline structure. Hereunder, I will subject to further discussion the above findings of the eggs of various parasites under a polarization microscope and their crystallographical properties and characteristics.

The shells of the eggs of ascaris show the highest stability, with their thick shells of crystalline structure showing strong double refraction and ellipsoid form. Upon swelling and separating the outermost layer by Ida's method, it was found that the next layer was the thickest and showed the highest degree of double refraction, greatly contributing to the stability of the shell. The second layer was found to have radially arranged crystalline structure, indicating its presumably high crystallizing aptness as nucleus. In general, it is of course necessary that an adequately powerful supporting basis is present, if a crystal is to grow to a size. From such a view-point it is needless to emphasize that a fertilized egg of ascaris with very high stability and with a thick layer of crystalline substance

in its shell offers an ideal nucleus in crystal formation. The presence of an albuminoid layer is a characteristic peculiar to an egg of ascaris not shared by an egg of any other parasite. Its uneven surface of large superficial area, its high cohesiveness and easy stainability are important factors in performing its duty of protecting the egg. They also conform with its aim at multiplying the chance of infection by adhesion to external objects. Its propensity to invite pigmentation and under definite circumstances to receive precipitation of crystals has been proven in the above described experiment. In this case, the albuminoid membrane is preserved to play the part of the skeleton of the forming crystals. The crystallographic properties ascertained in the above study are clearly of the nature that will, if a groundwork favoring a growth of crystals exists in the medium, lead to a growth of crystals too.

The unfertilized eggs of ascaris, however, have thin shells with only low double refraction, showing a perceptible difference from the fertilized eggs and indicating a probable frailty. It may be supposed that a participation of calcium and other inorganic components has a considerable influence in forming the fertilization membrane.[13] Such properties of an unfertilized egg probably make it worse adapted to form a nucleus of a bile stone than a fertilized egg, even if laid in the bile duct and the bile had acquired the predisposition facilitating the stone formation. Of course, the difference in the pictures of an unfertilized and a fertilized eggs under a polarization microscope offers a means for differentiating the two.

The eggs of hookworm and of trichostrongylus orientalis have only thin shells of low double refraction indicating a frail structure like the unfertilized eggs of ascaris. This property seems to be of service, when the early-grown youngs hatch out of the shells. Generally speaking, these parasites may have some chances to invade the biliary duct, and even if some of them enter the duct, they are not so apt to cause its stasis. Since the properties of their eggs are much inferior to fertilized eggs of ascaris for making nuclei of crystals, as stated above, the part such eggs play in pigment chalk stone formation must be nothing important.

The eggs of trichocephalus trichiurus are of a form and structure ensuring comparatively high stability. Their shells are thick and are composed of three layers, the outermost of which is readily stainable with pigments and the inner two show a comparatively high-degreed double refraction. It is of interest that the parts in the shell at the stopper-like projections which have been hitherto deemed to be shell-less and weak in resisting external force, are found to be provided with crystalline shell layers on their insides. As a biological observation, by the way, it is of some interest that the eggs of ascaris and trichocephalus trichiurus,

of which the hatching time is protracted, have comparatively stable forms, while those of trichostrongylus orientalis and hookworm with shorter hatching periods are more fragilly formed.

The eggs of clonorchis sinensis have thick shells of high double refraction. Their stable nature seems to indicate their possible serviceability as nuclei of biliary stones. Ifuku *et al.*,[14] have observed cases of distoma eggs forming the nuclei in biliary stones, and similar cases have been observed also in this clinic. Maki[15] has pointed out that the comparatively low frequency of distomatous bile stones in spite of the rather frequent distoma parasitism in Japan is probably due to the fact that infestation by distoma does not necessarily cause stasis of the biliary passage as do the ascaris.

Conclusion

1. The shells of the eggs of human parasites, such as ascaris lumbricoides, hookworm, trichostrongylus orientalis, trichocephalus trichiurus and clonorchis sinensis are crystalline in structure. Of these, the fertilized eggs of ascaris are the stablest.

2. The second from the outside is the stablest of the four layers of the ascaris egg-shell. The crystals in this layer are arranged radially.

3. The shells of the eggs of hookworm and trichostrongylus orientalis, which hatch rather early, show the lowest degree of double refraction and are frail in their crystallographic structure, the eggs of trichocephalus trichiurus and clonorchis sinensis coming next. The former have crystalline shell substance also under the stopper-like projection at their ends.

4. The albuminoid membrane covering the egg of ascaris may become a basis to initiate precipitation of calcium compounds when putrefied. This fact, coupled with the crystallographically stable structure of the shell, gives the highest importance of all foreign matters to be found in human bile to fertilized ascaris eggs as nuclei of biliary stones.

The author mentions with thanks that Assist. Prof. G. Yamamoto of the Biological Laboratory, Tohoku University, has accorded valuable suggestions in this study.

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Crystallographic Studies on the Eggs of Various Human Parasites

II. Spectroscopy of Fertilized Ascaris Egg Shells

By

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Introduction

In my 1st report, I have described my observations of the eggs of various intestinal parasites under a polarization microscope and have stated that the shells of the fertilized eggs of ascaris lumbricoides were found to occupy the position of highest importance in offering nucelar substance for gall stone formation. The elements constituting such egg shells were further followed up spectroscopically, and the important role such eggs may play as nucleus in the mechanism of biliary pigment stones has been clearly established. I will hereunder speak on the findings obtained in this study and give some discussing considerations on the results.

Experimental

Methods

About 1 cc. of intraoviduct fertilized eggs of ascaris lumbricoides was collected and washed repeatedly in a test tube with distilled water for 3 to 4 times. Care was taken to collect only fertilized eggs as far as possible, yet we could not exclude some unfertilized eggs being mixed. These eggs were then put in a cleansed mortar and crushed to the extent the egg shells were broken and the contents were released. The crushed eggs were next washed again in a cleansed and dried pointed glass, stirring and centrifugal sedimentation repeated 4–5 times with thorough care. When the last centrifugation was completed, the upper part was found to be composed of a mucous fluid apparently of albuminoid membranes mixed with a part of the egg contents, the medial part, mainly of sus-

pended egg shells and the lowest part, of the other part of the egg contents with impurities. This medial part containing much of the egg shells was subjected to qualitative spectral analysis (Shimadzu Quartz Spectrograph QF-60). Consequently, it must be admitted that the results are tainted by admixture of some impurities, but they may serve in indicating the approximate composition of the egg shells.

Results

Four rounds of experiments were made with fertilized eggs extracted from different ascaris each round. The wave-lengths of the lines appearing in the spectra of the specimens were of course determined with reference to the known wave-lengths of the spectral lines of iron as usual and special care was taken to collate the lines of copper with those of spectrum of copper sulfate. The composition of egg shells thus analysed was nearly constant in all the specimens, only some slight inequality in the tone of some lines being perceptible.

The elements composing the fertilized ascaris egg shells and the wave-lengths of their spectral lines, according to the results of these experiments, are listed below.

1) Ca	3158.9		2) Mg	2779.1	
	3179.3			2781.4	
	3359.3			2795.5	(U3)
	3933.7	(U1)		2802.7	(U2)
	3968.5	(U2)		2852.1	(U1)
	4225.4			3936.5	
	4226.7				
3) Si	2506.9		4) Fe	2382.0	
	2524.1			2395.6	
	2528.5			2599.4	
	2881.6	(U1)		2749.3	
				2755.7	
5) Cu	3247.5	(U1)	6) P	2534.0	
	3274.0	(U2)		2535.6	
	3370.4			2553.3	
				2554.9	

(In one specimen, the appearance of the spectral lines of P was somewhat doubtful.)

Discussion

In the past studies on the mechanism of gall stone formation, the physical and the chemical changes of their media have been discussed from various angles, but the search for the possible nucleus required for such a formation has been rather neglected. With regard to the gall stone

problem in Japan, it is very important, as stated in my 1st report, to pay our particular attention to this fact. We have been emphasising the importance of parasite eggs and their fractures, as they are often met with in Japan as nuclei in the formation of pigment chalk stones. My 1st report has been written from this stand-point, and this report is its sequel. In the experiments described herein, the materials entering in the composition of ascaris egg shells have been determined and compared with those composing the pigment chalk stones.

We cannot make a sweeping statement on what properties are apt to cause precipitation, but we may point out that a homogeneity of the nuclear substance to the medium is one of such properties. Upon spectra analysis of 13 specimens of pigment chalk stone and cholesterol pigment chalk stone, Takino[1] has detected 29 positive elements, of which he enumerated the 5 elements of Ca, Cu, Fe, Mg and Na as constantly recurring. Nishimura[2),3)] has detected 23 positive elements in gall stones, excluding pure cholesterol stones, of which he enumerates the 8 elements of Ca, Mg, Cu, Al, Mn, Fe, Si and Ti as constantly present and attributes particular meaning to Cu, Ca and Mg among them. Ishiyama[4)] and Mukoi[5)] also have published similar experimental results, but points out P as an occasionally detectable positive element. We could not determine the quantitative distribution of the compounding elements from these experiments, but have found the 6 elements of Ca, Mg, Si, Fe, Cu and P as traceable in the fertilized ascaris eggs, and these are in approximate coincidence with those constantly found in pigment chalk stones. This fact suggests that such egg shells have suitable property as nuclear substance for precipitation and growth of gall stone substances, whether the formation goes on in a stratified structure, a stratified plus radial structure or an amorphous structure. These elements are naturally identical in most cases with those composing the bile, in particular, the inflammatory bile, and thus the presence of the same elements in the ascaris eggs cannot be by itself a key to explain the whole mechanism of gall stone formation, but at least it may serve in interpreting the formation of pigment chalk stones prevalent in Japan, when considered together with the contamination of the bile passage by ascaris and their eggs and the physico-chemical changes of the bile caused in this system by such contamination.

Summary and Conclusion

1. Fertilized ascaris egg shells were spectrographically analysed and the component elements were found to be Ca, Mg, Si, Fe, Cu and P.

2. These compounding elements are nearly identical with those composing the pigment stones, and from this, it may be concluded that the fertilized eggs of ascaris have a significance not to be overlooked as

nuclei for pigment chalk stone formation.

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A Clinical and Experimental Study on the Management of Portal Vein during Pancreaticoduodenectomy

I. An Experimental Study on Transplantation of Portal Vein

By

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With the recent advances of cardiovascular surgery, many studies on the management of blood vessels in various diseases have been reported. They are, however, mainly on the work of transplantation of artery. It is probably the indication of transplantation or anastomosis of vein has not been frequently encountered. In cases of carcinoma of the head of the pancreas and carcinoma of the bile duct, carcinomatous infiltration frequently extends to the portal vein (Table I). Radical operation of the

TABLE I

Site of Infiltration~Adhesion in Cases of Pancreatoduodenectomy

Carcinoma of the head of the pancreas	0	1	2	3	2	5
Carcinoma of the bile duct	2	2	0	0	0	1
Chronic pancreatitis	2	2	0	0	0	2
Carcinoma of the pancreas accompanied with gastric carcinoma	0	5	1	1	0	0

V. p.: V. portae. V.l.: V. lienalis. V.m.s.: V. mesenterica superior.

lesion becomes impossible without operation on the portal system. The primary ligation and section of the portal vein is not allowable except under certain circumstances. Whenever end to end anastomosis of the portal vein and anastomosis of splenic vein with superior mesenteric vein is always

possible after carcinomatous infiltration to the portal vein is resected, there is not too much to worry about. We encountered the necessity to perform transplantation of grafts to the damaged portal vein after the wide defect of the vessel could not be sutured together. The author carried out experiments on transplantation of portal vein with various methods to find a clue to solve the method to deal with the injured vessel and attempted to apply the results on clinical cases.

Experimental

Methods and Results

Mongrel adult dogs were used and the operation was carried out entirely aseptic. After pancreatico-duodenal vein was incised between ligatures at the main branch of portal vein, the portal vein was excised about 5–15 mm between the site of pyloric vein and inferior mesenteric vein, and various methods of vessel transplantaton with various kind of grafts were used. The length of grafts were 14–25 mm. (Table II). Anastomosis were done with circular anastomosis using Moure's 2 supporting stitches or Carrel's 3 supporting stitches, and infrequently mattress sutures were used. Period of time for transplantation was between 4–49 minutes, average 21 minutes. Defect of retroperitoneum was not sutured and

Table II

Experiments on Transplanlation of the Portal Vein

(Appendix: Simple Anastomosis of Blood Vessel)

Grafts	Results	Cases	Average occlusion-time of portal flow	Period of observation
70% alcohol preserved artery	Survival Died	0 3	45 min.	6–24 hrs.
70% alcohol preserved vein	Survival Died	0 3	32 min.	12–18 hrs.
Fresh auto-vein	Survival Died	0 2	31 min.	10–17 hrs.
70% alcohol preserved vein tied on both edges with poly-ethylen tube	Survival Died	7 5	7 min. a. 30 sec. 15 min.	16–71 Days 7–30 hrs.
Fresh auto-vein (temporary occlusion of A. mesenterica superior)	Survival Died	3 2	24 min. 31 min. a. 30 sec.	5–23 Days 6–10 hrs.
(App.) Reanastomosis of portal vein after incision	Survival Died	3 0	16 min. a. 20 sec.	9–16 days

other organs were left intact. Anticoagulant was not administered postoperatively, and transfusion and fluid were also not given to the dogs.

1. 70% alcohol preserved aortic homografts (Table III)

TABLE III

70% Alcohol Preserved Aortic Homografts

No. (dog)		26	2	3
Weight (kg.)		12.4	14.0	13.2
Graft	Name	Thoracic aorta	Ditto	Ditto
	Period of preservation (days)	113	86	128
	Diameter (m.m.)	8.0	7.5	8.0
	Length (m.m.)	18.0	21.0	23.0
Diameter of portal vein (m.m.) (Site of transplantation)		8.0	8.5	7.5
Length of excized portal vein (m.m.)		10.0	7.0	15.0
Method of anastomosis		Moure's 2 supporting stitches	Ditto	Ditto
Time of occlusion of portal circulation (min.)		49	41	45
Congestion of small intestine during occlusion of portal vein		Servere	Ditto	Ditto
Release of obstruction of portal vein	Recovery of congestion	Rapid	Rather slow	Ditto
	Haematoma in wall of small intestine	Many	Ditto	Great many
	Petechiae in mesenterium	Great	Ditto	Ditto
Time till death		7 hrs.	6 hrs.	24 hrs.
Autopsy findings	Thrombus at anastomosis	(−) Complete Occlusion	(−) Ditto	(−) Ditto (White thrombus)
	Bleeding in the digestive tract	Moderate	Ditto	Great
	Formation of collaterals	(−)	(−)	(−)
	Ascites	Little	Ditto	Ditto
Cause of death and others		Shock	Shock	Shock. Tarry stool twice

Method: Following the vessel transpantation of Kimoto,[1] 70% alcohol preserved homografts of thoracic aorta were anastomosed with special made fine silk. Time of anastomosis was between 41 minutes and 49 minutes, average 45 minutes. After the anastomosis was completed, the clamp on the hepatic side was first released to expel the air and examine the leakage from the anastomosis. Then the clamp on the intestinal side was removed. Small bleeding from the anastomosis was stopped by leaving a piece of gauze on the anastomotic line temporarily.

Results: 3 were done. All died within 24 hours. In many cases, pyloric vein of the dog entered the splenic vein. After splenic vein was ligated, most portion of the great curvature of the stomach and cardia of the lesser curvature became congested, and the spleen also became engorged with blood. The occulsion of portal circulation caused intestinal congestion in a severe degree during operation, and haematoma in the intestinal wall, which caused loss of blood into the intestinal tract in several cases. After release of the clamp, congestion disappeared rapidly. Petechiae, however, were seen on the intestinal wall extensively in a pin size to a pea size. Thrombus was found at the anastomosis in all cases postoperatively, and the intestine were congestive in a severe degree. No. 3 showed white thrombus.

2. 70% alcohol preserved vein homografts (Table IV)

TABLE IV

70% Alcohol Preserved Venous Homografts

No. (dog)		6	27	28
Weight (kg.)		8.0	11.5	11.0
Graft	Name	V. iliaca communis	Ditto	V. jugularis externa
	Period of preservation (days)	9	13	18
	Diameter (m.m.)	7.5	7.5	7.0
	Length (m.m.)		21.0	21.0
Diameter of portal vein (m.m.) (Site of transplantation)		7.0	7.5	7.5
Length of excized portal vein (m.m.)		10.0	11.0	12.0
Method of anastomosis		Moure's 2 supporting stitches	Ditto	Ditto
Time of occlusion of portal circulation (min.)		32	34	30
Congestion of small intestine during occlusion of portal vein		Severe	Ditto	Ditto
Release of obstruction of portal vein	Recovery of congestion	Rapid	Rather slow	Ditto
	Haematoma in wall of small intestine	A few	Many	A few
	Petechiae in mesenterium	Moderate	Great	Moderate
Time till death		12 hrs.	18 hrs.	13 hrs.
Autopsy findings	Thrombus at anastomosis	(−) Complete occlusion	(−) Ditto	(−) Ditto
	Bleeding in the digestive canal	Slight	Moderate	Slight
	Formation of collaterals	(−)	(−)	(−)
	Ascites	A little	Little	Ditto
Cause of death and others		Shock	Shock	Shock

Method: 70% alcohol preserved common iliac vein and jugular veins were used.

Results: Thrombosis formed early and congestion of organs distal to portal vein occurred in a severe degree with 100% mortality. No bleeding was found at the graft.

3. Fresh vein autografts (Table V)

TABLE V

Fresh Auto-Vein

No. (dog)		7	9
Weight (Kg.)		10.5	12.3
Graft	Name	V. iliaca communis	Ditto
	Diameter (m.m.)	6.5	6.5
	Length (m.m.)	18.0	20.0
Diameter of portal vein (m.m.) (Site of transplantation)		7.0	7.5
Length of excized portal vein (m.m.)		9.5	11.0
Method of anastomosis		2 support mattress cont.	2 support over and over. cont.
Time of occlusion of portal circulation (min.)		34	28
Congestion of small intestine during occlusion of portal vein		Severe	Ditto
Release of obstruction of portal vein	Recovery of congestion	Rapid	Ditto
	Haematoma in wall of small intestine	Many	A few
	Petechiae in mesenterium	Great	Moderate
Time till death		17 hrs.	10 hrs.
Autopsy findings	Thrombus at anastomosis	(+) Complete occlusion	(+) Ditto
	Bleeding in the digestive canal	Moderate	Slight
	Formation of collaterals	(—)	(—)
	Ascites	Little	A little
Cause of death and others		Shock	Shock

Method: Common iliac vein was removed aseptic from the dog and thrown into physiologic saline solution. The abdomen was opened with a different incision and the vein was anastomosed into the defect of the portal vein with 2 supporting stitches and one case with mattress suture.

Results: All died in shock. Autopsy findings showed congestion in the organs distal to the portal vein. Thrombus was found in the graft. No bleeding nor ascites was recognized at the transplanted site.

4. Polyethylene tube with 70% alcohol preserved vein homograft (non-suture method) (Table VI)

TABLE

70% Alcohol Preserved Vein Tied on

No. (dog)		1	4	5	8
Weight (kg.)		13	7.5	12.5	9.5
Graft	Name	V. iliaca communis	Ditto	Ditto	Ditto
	Period of preservation (days)	5 (hrs).	4	5	4
	Diameter (m.m.)	7.0	7.0	7.5	7.5
	Length (tied with P-tube) (m.m.)	17.0	25.0	20.0	19.0
P-tube	Diameter (inside) (m.m.)	8.0	6.0	6.0	6.0
	Thickness (m.m.)	0.8	0.8	8.8	0.8
	Length (each) (m.m.)	5.0, 5.0	5.0, 5.0	5.0, 6.0	4.0, 5.0
Diameter of portal vein (m.m.) (Site of transplantation)		9.0	8.0	8.5	8.0
Length of excized portal vein (m.m.)		8.0	10.0	7.0	7.0
Time of occlusion of portal circulation (min.)		40	14	18	8
Congestion of small intestine during occlusion of portal vein		Severe	Tolerable	Severe	Slight
Release of obstruction of portal vein	Recovery of congestion	Rapid	Ditto	Rather slow	Rapid
	Haematoma in wall of small intestine	Many	None	Few	None
	Petechiae in mesenterium	Great	Few	Ditto	None
Time till death		32 hrs.	71 days	12 hrs.	16 days
Autopsy findings	Thrombus at anastomosis	(−) Complete occlusion	(−) Ditto	(−) Ditto	(−) Ditto
	Bleeding in the digestive canal	Moderate	None	Moderate	None
	Fibrosis around P-tube	(−)	(+)	(−)	(+)
	Formation of collaterals	(−)	(+)	(−)	(+)
	Ascites	70 cc	Little	Ditto	None
Cause of death and others		Shock. Tarry stool once	Shock. Tarry stool after operation	Shock. Stop respiration for a time. Artificial respiration for 6 min.	Sacrificed

Method: Polyethylene tube (called P-tube afterwards) were made following John's technique[2] with glass tubes. They were made 1 mm. thick and cut 0.4–0.5 cm. long as shown in Fig. 1 with markings in the middle to stop the slip after the inserted vein graft were tied together. Vein grafts (chiefly common iliac veins) were taken from a dog immeditate after death and inserted through the P tube and turned out-side as a cuff with tie on both edges. Silk was used to tie it and prepared grafts were

VI

Both Edges with Polyethylen Tube

10	11	12	13	14	15	16	17
16	7.7	15	14	14.8	11.3	12.6	14.7
V. cava inferior	V. iliaca comm.	Ditto	Ditto	Ditto	Ditto	Ditto	Ditto
3	11	12	22	18	16	42	31
12	7.0	7.0	7.0	7.0	7.0	7.5	9.5
25.0	18.0	20.0	21.0	23.0	21.0	24.0	20.0
10.0	6.0	6.0	6.0	6.0	6.0	6.0	8.0
0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
6.0, 6.0	4.0, 5.0	5.0, 5.0	5.0, 5.0	4.0, 5.0	5.0, 5.0	5.0, 5.0	5.0, 5.0
11.0	7.5	8.5	7.5	7.5	8.0	8.5	9.5
13.0	8.0	8.0	7.0	5.0	8.0	8.0	5.0
4 min. a. 30 sec.	6	6	4	7	8	6 min. a. 30 sec.	6
Ditto	Tolerable	Slight	Ditto	Ditto	Tolerable	Slight	Ditto
Ditto	Ditto	Ditto	Ditto	Ditto	Ditto	Ditto	Ditto
Ditto	Ditto	Ditto	Ditto	Ditto	Ditto	Ditto	Ditto
Ditto	Ditto	Ditto	Ditto	Ditto	Ditto	Ditto	Ditto
8 hrs.	28 days	20 days	47 days	7 hrs.	21 days	18 days	12 hrs.
(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)
Ditto	Ditto	Ditto	Ditto	Ditto	Ditto	Ditto	Ditto
A little	None	Ditto	Ditto	A little	None	Ditto	Moderate
(−)	(+)	(+)	(+)	(−)	(+)	(+)	(−)
(−)	(+)	(+)	(+)	(−)	(+)	(+)	(−)
Ditto							
Shock	Ditto Sacrificed	Little Ditto	None Ditto	Ditto Shock	A little Sacrificed	None Ditto	Ditto Shock

preserved in 70% alcohol. 3 different kind of P tubes, i.e., 6,8, and 10 mm were made according to the diameter of the portal vein. After the vein grafts were inserted into the P-tube and cuffed over the edges, the length of the graft stretched a few mm. longer than the original length. After the portal vein was excised, the prepared P tube was slipped into the hepatic side first, and tied with simple ligature or such ligature as shown in Fig. 1 (5). 10% sodium citrate was filled in the P tube and slipped

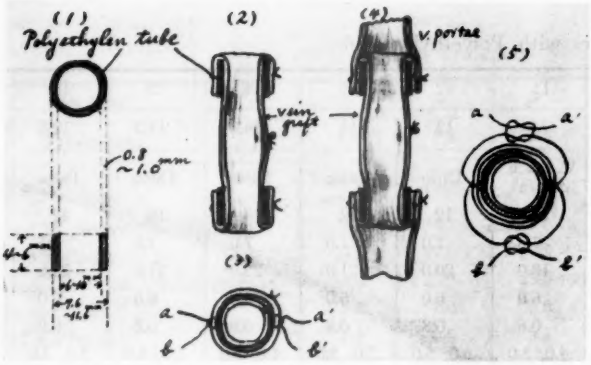


Fig. 1. Scheme of the method of ligation and insertion of the vein graft into the polyethylene tube.

and tied on the intestinal side as the same.

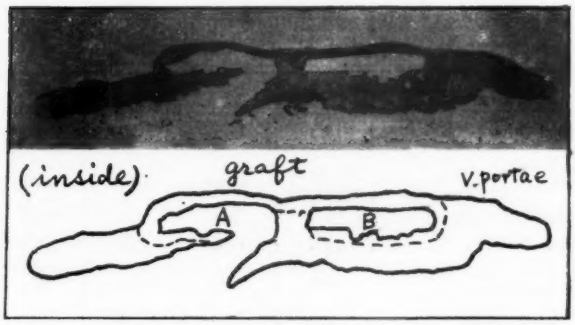
Results: As shown in Table VI, the prepared P tube used in No. I was bigger than the portal vein which required 40 minutes to complete the procedure. The other grafts, however, could be finished within 4–18 minutes. No cases had bleeding at the anastomosis during the period of observation. No. 5, 10, 14 & 17 all died in the condition of shock. The survival time, however, was prolonged more than that in part 1 experiment. At autopsy, thrombus at the site of transplantation was found, and severe degree of intestinal and splenic congestion were both observed. No. 14 had bleeding in the intestinal tract. Among 12 cases, 7 survived. At autopsy fibrosis was found around the transplanted P tube, and thrombus in the graft caused complete occlusion of the portal vein. Many collaterals were seen without congestion in the organs distal to the portal vein. Those which survived long enough did not have remarkable ascites.

Pathohistological findings: At the line of anastomosis, intima covered the thrombus which was already under organization and also the thin layer of thrombus over the graft (Fig. 2 b). The graft contracted longitudinally and the P tube approached together. In Fig. 2 a, the P tube is transparent. The staining of the cell nuclei of the graft is bad and haemosiderin is observed. Fibrosis is seen around the tube (Fig. 2 c) and invasion of lymphocytes is seen as a foreign reaction. As thrombus has dropped during the preparation of this specimen, the inside looks smooth (Fig. 2 a, b).

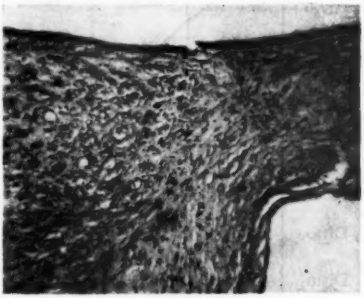
5. Fresh vein autografts (Table VII). (Temporary occlusion of superior mesenteric artery)

Method: Blood flow of superior mesenteric artery was temporary occluded with a bulldog clamp and it was removed soon after the trans-

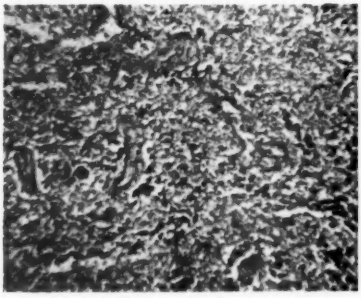
Fig. 2



a. A vertical section of transplanted graft A & B: P-tubes are transparent. H.E. ×9.



b. Site of anastomosis. Right side: wall of graft. Left side: wall of portal vein in host. Right, inferior and white part: wall of of P-tube H. E. 10×8.



c. Fibrosis. H. E. 10×8.

plantation of grafts of portal vein was completed. The time required for transplantation was 20–35 minutes, average 26 minutes.

Results: After the occlusion of superior mesenteric artery, the intestine became anemic and slight congestion of the intestine was noticed during transplantation. The intestine, however, became bright red soon after the opening of blood flow in the superior mesenteric artery without any petechiae and signs of congestion, and the intestine vigorously regained its peristalsis. Of 5 cases, 3 survived. Of 2 fatal cases, 1 (No. 20) died by considerable blood loss due to unrecognized splenic rupture during operation and the other one (No. 19) died due to stenosis after completion of the anastomosis, and early thrombus formation caused obstruction of portal vein. Of 3 survival cases, 2 cases showed complete obstruction by thrombus at the site of transplantation, and 1 case (No. 18) died on the

TABLE VII

Fresh Auto-Vein
(Temporary Occlusion of A. Mesenterica Superior)

No. (dog)		18	19	20	22	23
Weight (kg.)		13.5	14.5	8.0	6.5	7.0
Name		V. iliaca communis	Ditto	Ditto	Ditto	Ditto
Graft	Diameter (m.m.)	7.0	8.0	6.0	6.0	6.0
	Length (m.m.)	14.0	16.0	23.0	21.0	22.0
Diameter of portal vein (m.m.) (Site of transplantation)		8.0	10.0	8.0	6.5	7.0
Length of excized portal vein (m.m.)		6.0	7.0	15.0	8.0	8.0
Method of anastomosis		Moure's supporting stitches	Ditto	Ditto	Carrel-stitch	Carrel-stitch
Time of occlusion of portal circulation (min.)		20	22	35	24	28
Time of occlusion of a. mesenterica superior		22	23	40	23	30
Congestion of small intestine during occlusion of portal vein		Tolerable	Slight	Tolerable	Slight	Ditto
Release of obstruction of portal vein	Recovery of congestion	Rapid	Ditto	Ditto	Ditto	Ditto
	Haematoma in wall of small intestine	None	Ditto	Few	None	Ditto
	Petechiae in mesenterium	None	Ditto	Slight	None	Ditto
Time till death		5 days	10 hrs.	6 hrs.	23 days	20 days
Autopsy findings	Thrombus at anastomosis	(+) Partially patent	(+) Complete occlusion	(+) Ditto	(+) Ditto	(+) Ditto
	Bleeding in digestive canal	None	Ditto	Ditto	Ditto	Ditto
	Formation collaterals	(+)	(−)	(−)	(+)	(+)
	Ascites	A little	Little	None	Ditto	None
Causes of death and others		Intussusception	Shock	Shock Splenectomy	Sacrificed	Ditto

5th day due to intestinal obstruction. Only slight amount of ascites was seen and the graft was patent with narrow lumen. All 3 cases showed a large amount of collateral circulation.

Appendix: Incision of portal vein with immediate anastomosis (Table VIII)

Method: Portal vein was cut and immediately resutured again. During the anastomosis, the blood flow was temporarily occluded, and

TABLE VIII

(App.) Reanastomosis of Portal Vein After Incision
(Temporary Occlusion of A. mesenterica superior)

No. (dog)		21	26	29
Weight (kg.)		12.0	13.2	11.0
Diameter of portal vein (m.m.) (Site of anastomosis)		7.5	8.0	7.5
Method of anastomosis		Carrel-stitch	Carrel-stich	Carrel-stitch
Time of occlusion of portal vein (min.)		18	15	16
Time of occlusion of a. mesenterica superior (min.)		20	17	18
Congestion of small intestine during occlusion of portal vein		Slight	Ditto	Ditto
Release of obstruction of portal vein	Recovery of congestion	Rapid	Ditto	Ditto
	Haematoma in wall of small intestine	None	Ditto	Ditto
	Petechiae in mesenterium	None	Ditto	Ditto
Time till death		9 days	16 days	21 days
Autopsy findings	Thrombus at anastomosis	(−) Stenosis	(−) Ditto	(+) Partially patent
	Bleeding in the digestive canal	None	Ditto	Ditto
	Formation of collaterals	(+)	(+)	Ditto
	Ascites	A little	None	(+)
Cause of death and others		Sacrificed	Ditto	Ditto

released right after anastomosis. 3 cases were done with Carrel's method.

Results: 3 cases all survived. At autopsy, no remarkable thrombus was seen at the anastomosis with good patency of the portal vein. Good collaterals were seen without congestion of the intestine and accumulation of ascites.

COMMENT AND SUMMARY

Transplantation of blood vessels has been carried out since Carrel,[3] and chiefly vein grafts were used to bridge the defect of artery in clinical cases. Recently, operation on portal vein and superior and inferior vena cavae have been attempted, and transplantation of vein grafts has been tried. The results, however, have been unsatisfactory. Especially, transplantation of portal vein hasn't yet been reported. The problems of transplantation of arterial grafts have been intensively investigated by Gross, *et al.*,[4] while Kimoto,[1),5] and Toda[6] in Japan have reported excellent results in the annual report on blood vessel surgery at the general meeting of the Japan Surgical Society. It has been known that arterial grafts were better than vein grafts to replace the arterial defect. The

accessiblility of the grafts became an important problem. Therefore, an intensive investigation of the preservation of homo- and heterografts have ensued. In order to preserve the grafts in a living condition, Gross[4] used B.S.S. solution, and Tyrode solution added with serum, while Swan[7] used Ringer's solution added with 10% serum. The grafts, however, could be only used within 40 days after preservation with satisfactory results. These grafts should be taken out aseptic from the body within 5–6 hours after death. The age of the body should be within 3–40 years old and died by trauma. The grafts are also required to be kept 4 degrees C. in a refrigerator. In order to overcome these complicated methods, Peirce and Gross[8] used 4% formalin, Paulocci,[9] pure alcohol, and Deterling,[10] frozen grafts. These dead grafts were used in the animals. In Japan, Kimoto *et al.*,[1] used 70% alcohol preserved grafts with high degree of success in experimental and clinical cases. Gross,[4] Swan,[7,11] Sauvage,[12] Deterling *et al*,[13] demonstrated that these homografts could be used with good results, while heterografts fixed in solution could only be safely used[14,15] in spite of poor results with fresh heterografts.[4]

The author used 70% alcohol preserved artery and vein homografts which have shown good results clinically in Japan to transplant the portal vein. All cases except those using non-suture method, and temporary occlusion of superior mesenteric artery were unsatisfactory as was expected. Analyzing these failures, the following points should be considered. (a) Occlusion of the portal vein for 30–40 minutes easily induces irreversible shock, (b) 70% alcohol preserved grafts tend to become thrombosed easier than the artery with high blood pressure and fast blood flow, (c) anastomosis is required on both edges which brings many threads inside the blood vessel and thrombus forms easily. Fatal cause is due to a large amount of blood shunted into the organs distal to the occulusion of portal circulation. I is said that one hour is the maximum permissible time for occlusion of the portal vein.[16] Moore[17] reported a case with occlusion for 45 minutes without ill effects. We experienced two cases with occlusion for 30 minutes without complications. In order to prevent a large shunt of blood of the portal bed, (1) rapid anastomosis, (2) anastomosis during temporary occlusion of superior mesenteric artery, (3) maintenance of blood flow during anastomosis, are important factors to be considered. The first problem was solved by the method described in part 4. The dogs, however, died within 2–3 hours if thrombus formed early in the graft. It seems that dog can survive if the blood flow could be maintained for over 5 days, because the collateral circulation is formed within that period. It has been known that if portal vein occluded gradually it could survive regardless complete occlusion of the portal blood flow.[18] Ligation of portal vein can also be done safely with secondary or tertiary operation

on the portal vein.[18),19)] The reason that the successful results was obtained by the author is due to the considerations of the above factors.

The non-suture method by the author has already been introduced by Murphy[20)] and Payr[21),22)] for end-to-end anastomosis. Recently, Blakemore[23)-26)] *et al.* used vitallium tube. In Japan, Toda[6)] and Kimoto[27)] *et al.* tried prothese. They, however, occluded with thrombus eventually and the results have been poor. Johns[28)] compared continuous suture with non-suture method and reported that the former was successful in 72.7%, while the latter was 17.2% successful. He commented that these tubes remained in the tissue as foreign bodies, caused hard fibrosis and its lack of elasticity contributed to thrombus formation. Murray & James[29)] reported that when blood vessel is tied over metal tube, the wall of blood vessel becomes necrotic and bleeding occurrs. The author's case didn't show any bleeding.

Temporary occlusion of superior mesenteric artery as shown in part 1 was done to prevent the shunt of blood in the portal bed. No ill effects were shown during the time of occlusion. Nelson & Kremar[30)] reported that if blood flow of the portal bed is occluded in dog and cat for 4 hours, they would die, whereas in dogs, simultaneous occlusion of superior mesenteric artery and portal vein, the portal vein could be safely occluded for 2 hours. Moore[17)] reported two cases of simultaneous obstruction of portal vein and superior mesenteric artery for 40 minutes and 95 minutes without any serious complications. Temporary occlusion of superior mesenteric artery during anastomosis of portal vein seems to be useful for prevention of shock.

Though stenosis after anastomosis of vein cannot be avoided, no thrombus was found. Since old days, thrombosis is said to occurr easily after anastomosis of vein.[3),31),32)] The use of heparin is recommended.

During resection of carcinoma of the pancreas, Parson[33)] mentioned the necessity of excision of portal vein and superior mesenteric vein. Child,[34)-36)] Brunschwig,[37)] Dermott,[38)] Schafer[39)] and Moore[14),40)] have reported the method of managing with these problems. No perfect method, however, has been reported so far. Though the possibility of primary excision of the portal vein with blood vessel transplantation has been demonstrated by the author, its clinical application in cases of pancreaticoduodenectomy requires further studies. The author believes that its clinical application is expectful. With the present experiment, the portal blood flow is eventually maintained by the collaterals. Permanent maintenance of the portal circulation becomes the ultimate problem.

Conclusion

1. Transplantation of 70% alcohol preserved aorta and vein homo-

grafts into portal vein requires a long period of time. The animal tends to become shocked at the end of operation and thrombus forms in an early period after the operation. Their usage are prohibited. Aorta and vein grafts were used in 3 animals, respectively.

2. Transplantation of fresh vein grafts showed the same results as the alcohol grafts. 2 cases were subjected to the experiment with total death. When temporary occlusion of superior mesenteric artery was tried during the transplantation with fresh vein grafts (common iliac vein), the anastomosis tended to become stenotic. Of 5 cases, 3 survived.

3. Of 12 cases of polyethlene tubes inserted with alcohol preserved vein grafts and tied at both edges, 7 survived. The grafts all thrombosed and many collaterals were formed. Occlusion was due to pressure from the surrounding fibrosis and thrombus formation. These changes seems to occurr within 1–2 weeks. If the blood flow can be maintained during this period, the dogs can survive. It is believed that clinical application is warranted.

4. In dogs, the time of occlusion of portal vein becomes important. 20 minutes is safe, while 30 minutes is danger. In those cases with temporary occlusion of superior mesenteric artery, transplantation of portal vein was not accompanied with any danger.

5. If anastomosis of portal vein can be performed without tension, it can be safely done. 3 cases were all done with success. Permanent patency can also be expected.

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A Clinical and Experimental Study on the Management of Portal Vein during Pancreaticoduodenectomy

II. Study of Collateral Circulation after Occlusion of the Main Branch of Portal Vein

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As being reported in chapter I, most of the site of transplantation became thrombosed. The animals, however, survived by the formation of collaterals. This finding is coincident with Mizuno's report saying that in dogs, survival of the animal was possible by secondary ligation of the portal vein. According to Child, in a case of carcinomatous infiltration by carcinoma of the head of the pancreas, he was able to perform primary ligation of portal vein with survival. These reports together with the results of the author suggest that extensive operation on pancreaticoduodenectomy is promising in the future. In cases of obstruction of portal circulation, the study of collaterals toward the liver and other organs is an important problem whenever operation on the portal vein is contemplated. 13 cases of transplantation of the portal vein reported in part 1 and 2 cases of total obstruction of portal vein among 4 cases which were autopsied after pancreaticoduodenectomy were studied on the direction of the collaterals.

Collaterals of the Portal System in Dogs Described in Report I

Method: Laparatomy was performed by a midline incision. Gastrolienal vein (in dog, it is the vein on the greater curvature of the stomach including most part of the splenic vein and coronary veins) and superior pancreaticoduodenal vein were both cut between ligatures. The portal vein between the point of superior mesenteric vein where the inferior mesenteric vein enters (generally speaking, in dogs, the inferior mesenteric vein enters the superior mesenteric vein, and not into the gastrolienal vein as in human) and pyloric vein was completely freed. The ob-

struction occurred in the middle of the main branch of portal vein. Therefore, pyloric vein and the collaterals around the hepatic portal only remains. Venography by x-ray, postoperative gross findings and injection of pigment into the veins were used to study the collaterals.

Results: Collaterals and its incidence are described in Table I.

TABLE I

Collaterals (Obstruction of portal vein)

A. Collaterals towards the liver

(1) V. mesenterica superior—V. pancreaticoduodenalis inferior—V. pancreaticoduodenalis superior—V. portae (hepatic side of graft) (13/13)

(2) V. mesenterica superior—V. pancreaticoduodenalis superior and small veins around graft—venous plexus around bile duct and V. portae—V. portae (hepatic side of graft) or Ve. portae accessoriae. (13/13)

(3) V. gastrolienalis (the greater part) and V. coronaria ventriculi (a part)—V. pancreatica major—V. pylorica—V. portae (hepatic side of graft). (8/13)

(4) V. coronaria ventriculi (a part)—small veins in minor omentum—V. portae (hepatic side of graft). (7/13)

(5) (In adhesions between major omentum and mesenterium) veins of intestine and mesenterium—veins in major omentum (a part)—V. gastrolienalis—(3)—V. portae. (2/13)

B. Collaterals detouring the liver

(6) V. gastrolienalis (a part)—V. coronaria ventriculi or V. gastrica sinistra—Ve. esophageae. (13/13)

(7) Veins around minor omental sac and pancreatic tail—V. suprarenalis media or V. renalis propria sinistra—V. renalis sinistra. (7/13)

(8) V. mesenterica superior and V. mesenterica inferior—net of veins in mesocolon—V. spermatica sinistra and V. renalis propria sinistra—V. renalis sinistra. (11/13)

(9) V. gastrolienalis (a part)—veins in Lig. lienocolica—net of small veins in mesocolon—V. mesenterica inferior—(8). (4/13)

(10) V. haemorrhoidalis superior—V. haemorrhoidalis media—(V. hyogastrica)—V. cava inferior. (13/13)

(11) Net of small veins in mesocolon—small veins in retroperitoneum—V. cava inferior. (1/13)

(12) Small veins in mesocolon—small veins in retroperitoneum—V. spermatica dextra—V. renalis. (2/13)

(13) Small veins in mesenterium—small veins in retroperitoneum—V. renalis dextra. (1/13)

(14) Net of small veins in mesocolon of sigmoideum—small veins in retroperitoneum (or plexus venosus sacrales anteriores)—V. lumbalis ascendens (or V. iliaca communis). (4/13)

(15) (In adhesions between great omentum and abdominal wall) small veins of major omentum—veins of abdominal wall. (3/13)

(1), (2), and (10) were seen in all cases, and (7) & (8), described by Isobe[1] were frequently observed. (3) & (4) were both seen in many cases. Not a single case showed esophageal varix. Postopertively, No. 1 & No. 4 (26) showed intestinal bleeding. After several days, however, bleeding was not found in any case. Fig. 1 (A, B, C, D &E) shows the collaterals obtained by injection of 70% Diodrast into the gastrocoronary vein, gastro-splenic vein, superior mesenteric vein, inferior mesenteric vein, & inferior

Fig. 1

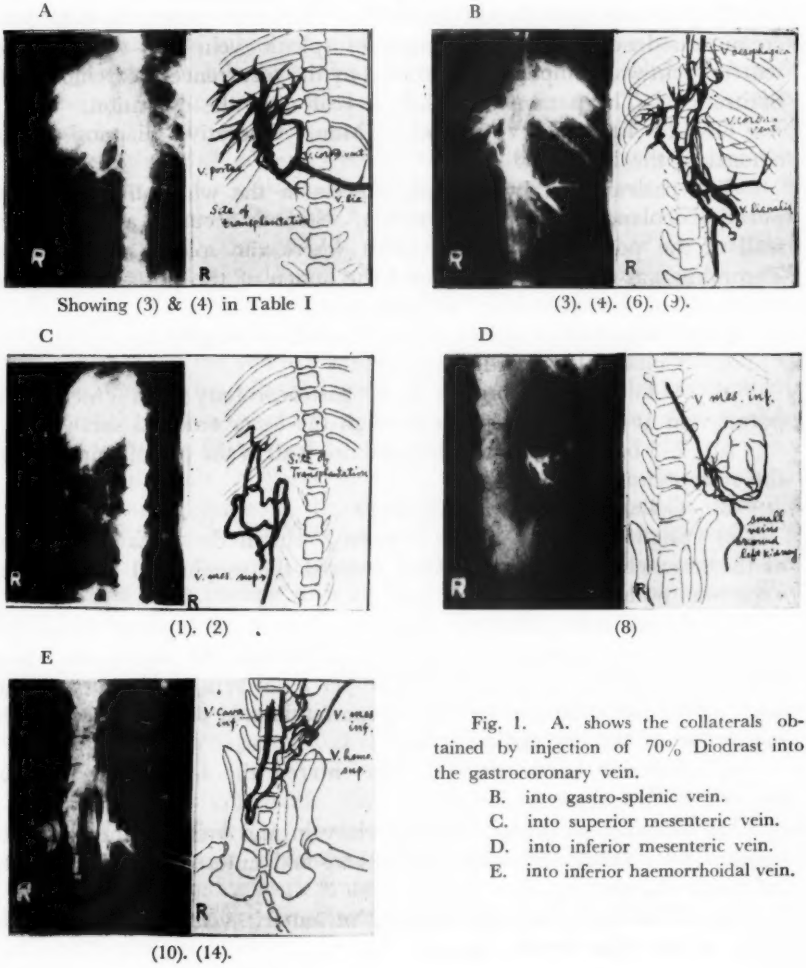

Fig. 1. A. shows the collaterals obtained by injection of 70% Diodrast into the gastrocoronary vein.

B. into gastro-splenic vein.

C. into superior mesenteric vein.

D. into inferior mesenteric vein.

E. into inferior haemorrhoidal vein.

haemorrhoidal vein, respectively.

Collaterals in Occlusion of Portal Vein in Human Beings

The collaterals in occlusion of portal vein were studied by macroscopic findings and injection of pigment. Those which showed obstruction of portal vein are described as follows:

Case 1. Harada. 48 years old. Woman. Operative diagnosis: carcinoma of the head of the pancreas.

The patient died by carcinomatous peritonitis occurring after pancreaticoduodenectomy. Anastomosis of splenic vein and superior mesenteric vein was completely obstructed by the recurrence of carcinomatous infiltration. The patient lived half a year after the operation.

Case 2. Endo. 52 years old. Man. Operative diagnosis: carcinoma of the bile duct.

The patient died by multiple abscess in the whole liver and suppurative cholangitis after the operation. Stenosis occurred at the sutured wall of the portal vein at the point where the splenic vein entered. Thrombus was found and obstructed the lumen of the portal vein. The patient lived 4 months after the operation.

Both cases showed ascites without intestinal congestion.

A) Collaterals toward the liver

a) A fairly large branch from gastrocoronary vein entered the portal vein at the hepatic porta through the lesser omental sac.

b) 2–3 branches entered the posterior wall of the portal vein through the retroperitoneum.

B) Collaterals detouring the liver

c) Slight dilatation of the coronary vein in the remaining portion of the stomach was noticed. They entered the esophageal vein and no varix was noticed in the esophagus.

d) Many new collaterals from the branch of superior mesenteric vein entered the inferior mesenteric vein through the mesocolon. New blood vessels which entered the left proper renal vein by the surrounding tissue of the left kidney or by the left spermatic vein were also seen in the mesocolon of the descending colon.

e) Superior haemorrhoidal vein entered the hypogastric vein by the middle haemorrhoidal vein.

f) Branches of superior mesenteric vein and veins in the retroperitoneum were connected with each other, and entered directly into the inferior vena cava.

g) Collaterals from the superior mesenteric vein entered the right colon of the right hepatic flexure.

h) Several veins which were slightly dilated were connected with the colon and great omentum which were firmly adhesed with the peritoneum.

i) No congestion was seen in the intestine. The intestine was rather atrophic.

Summary

The collateral circulation is established within 1–2 weeks in experimental animals after obstruction of the portal vein. These animals had no operation except on splenic vein and superior pancreatico-duodenal vein. In patients subjected to pancreaticoduodenectomy for carcinoma of the pancreas or bile duct, the collateral formation was relatively limited by resection of the tumor with half of the stomach, most part of the duodenum, half of the pancreas and common bile duct. As already being shown by Edwards,[2] accessory portal veins in human beings are important in establishment for collaterals toward the liver. It seems important to preserve the vessels around the hepatic portae as far as no carcinomatous infiltration exists. Though (12), (13) & (14) in table I are not described in the literature, they do not seem to play an important role as portal collaterals. Veins described by Retzius[1] is important when superior mesenteric vein is obstructed. (9) seems to have been built by the pressure difference of the bed of gastrosplenic vein and mesenteric veins during the operation. The collateral vessels (superior haemorrhoidal vein middle haemorrhoidal vein—venules in the pelvis-spermatic vein) described by Retzius were not discovered by the author.

The collaterals (a) Portal vein -paraumbillical vein in the abdominal wall, i.e. Caput Medusae; (b) Sappey's vein[3] -diaphragmatic veins did not appear. The reason that varix in the esophagus was not noticed is probably due to the small amount of blood flow in the esophageal vein, because the veins leading to it are cut at the operation when the portal vein is transplanted.

Conclusion

1. Collaterals in occlusion of portal vein was studied on the survival dogs described in chapter I and clinical cases autopsied after pancreaticoduodenectomy.

2. 2–3 new collaterals were described in experimental animals. Of them (12), (13) & (11) seems to play an important role in case of obstruction of superior mesenteric veins in human beings. In autopsied cases, these collaterals were found.

3. Collaterals are established 1–2 weeks after occlusion of portal vein in healthy animals.

4. No esophageal varix was found in both experimental dogs and clinical cases which underwent pancreaticoduodenectomy. Ascites were not remarkable in animals.

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A Clinical and Experimental Study on the Management of Portal Vein during Pancreaticoduodenectomy

III. Clinical Cases with Operation on the Portal Vein during Pancreaticoduodenectomy

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One of the most difficult problems during pancreaticoduodenectomy for primary carcinoma of the head of pancreas is the management of portal vein. Generally speaking, portal vein is surrounded posterior to the head of the pancreas and lies directly to the organ. Therefore, even though no carcinomatous infiltration to the portal vein is remarkable, the branch of portal vein is injured easily. When carcinoma infiltrates to the portal vein, the danger of injury to the vein becomes greater. If the indication for operation on carcinoma of the head of pancreas is strictly selected, infiltration to portal vein should be exceptional cases. The degree of infiltration to the portal vein of carcinoma of the head of the pancreas, however, cannot be easily decided even after laparatomy. Such infiltrations are found when pancreas is freed from the surrounding tissues toward the head of the organ after it is amputated at the body of pancreas. The degree of infiltration in 31 cases of pancreaticoduodenectomy during the past 5 years in the Prof. Katsura's Surgical Service of the Tohoku University Hospital is enumerated in Table I. All cases had variable degree of infiltration to the portal vein in carcinoma of the head of pancreas. At the earlier stage of pancreaticoduodenectomy, the operation was first performed from the duodenal side to see the degree of infiltration. This procedure, however, is accompanied with immenient danger of injury to the portal vein and the operation is replaced by removing the tumor from the tail of pancreas at the present time. Several cases with injury of portal vein, superior mesenteric vein, and the anastomosis of the vessels are described, and comments upon the surgery of the portal system from the standpoint of pancreatectomy are made in this report.

TABLE I

Rate of Infiltration and Adhesion to Portal System in 31 Cases

	Not invaded	Slight	Moderate	Severe	Total
Carcinoma of the head of the pancreas	0	4	5	4	13
Carcinoma of the bile duct	2	2	1	0	5
Chronic pancreatitis	2	2	1	1	6
Carcinoma of the pancreas accompanied with gastric carcinoma	0	6	1	0	7

Clinical Cases

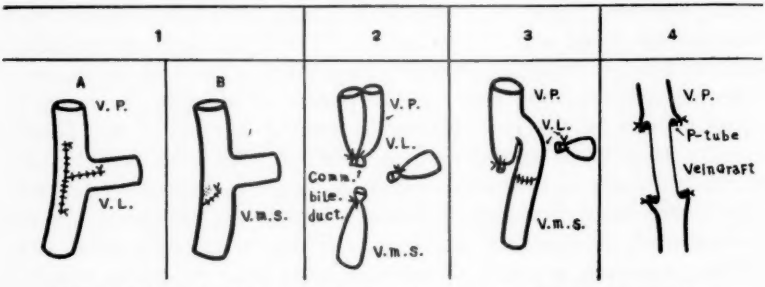
Injury to the portal vein with suture of the wall

A) Case 1. Endo. 52 years old, Man. Operative diagnosis: carcinoma of the bile duct.

Carcinoma occurred at the junction of the hepatic and cystic duct and extended to the posterior surface of the pancreas. During dissection of the pancreas, the portal vein was injuried just before the junction of splenic vein and superior mesenteric vein. The portal vein was also cut longitudinally by dissecting the carcinomatous infiltration which made the hole in the portal vein as letter T (Table II. 1, A). The defect was sutured laterally with temporary occlusion of the lumen. Despite transient

TABLE II

Scheme of 5 Clinical Cases with Operation on the Portal Vein



V.P.: V. portae. V.l.: V. lienalis. V.m.s.: V. mesenterica superior.

edema was noticed in the intestinal wall caused by narrowing of the blood vessel, the swelling disappeared at the end of operation. Postoperatively, the patient had continous haematemesis, tarry stool and gradual ac-

cumulation of ascites showing typical syndrome of portal hypertension. The patient, however, was discharged with improvement of general conditions. Unfortunately, he died 4 months later with multiple liver abscess. At autopsy, the lumen of portal vein was completely obstructed. The amount of ascites was 700 cc. The spleen weighed 375 gr. Collaterals were seen in abundance without esophageal varix. Causes for the postoperative haematemesis could not be clarified. Multiple liver abscess with cholangitis were noticed.

B) Case 2. Isozaki. 48 years old. Man. Operative diagnosis: chronic sclerosing pancreatitis.

As the pancreas was hard and felt like carcinoma by palpation, pancreaticoduodenectomy was performed. During dissection of the head of pancreas, great blood loss occurred by accidental injury to the superior mesenteric vein. Both ends were clamped and lateral suture of the blood vessel wall was performed (Table II, 1, B.). In spite of obstruction of blood flow lasted for 15 minutes, the intestinal wall became edematous without return to normalcy at the end of operation.

Cases with ligation of the portal vein

Case 1. Hayama. 56 years old. Woman. Operative diagnosis: carcinoma of the head of pancreas.

This case was operated in the early period of pancreaticoduodenectomy. During dissection of the tumor of the pancreas from the duodenal side, the portal vein was ligated with the dilated common bile duct without noticing the vessel lying behind it (Table II, 2). When edema was noticed on the intestine, splenic vein was already resected. Therefore, no anastomosis was able to perform. Expecting the formation of collaterals, the abdominal wall was closed. Though preoperative blood pressure was 102–76 mm. Hg., puls 60, the blood pressure could not be obtained at the end of operation, and the puls was 120, frequent and weak. She died an hour and a half after the operation. About 4 hours lasted after the ligation of the portal vein.

Case of end-to-end anastomosis between superior mesenteric vein and splenic vein

Case 1. Harada. 48 years old. Woman. Operative diagnosis: carcinoma of the head of pancreas.

The tumor was limited to the head of the pancreas. During dissection of the tumor in the head of the pancreas from the portal vein, the carcinomatous infiltration into the superior mesenteric vein was perforated. As anastomosis between blood vessels was impossible, the lesion including the vessel was resected about 2.5 cm, and the splenic vein was anstomosed with the superior mesenteric vein on the hepatic side. (Table IIa, 3). Postoperatively, no ascites were found. The patient was dis-

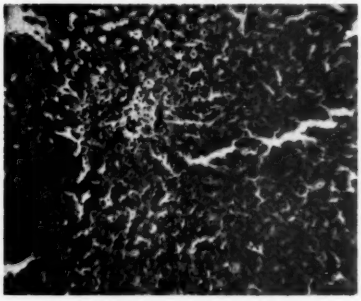
charged on the 50th postoperative day with normal liver function. Unfortunately, he died 4 months later with recurrence of carcinoma. At autopsy, peritonitis carcinomatosa was found with 4,000 cc. of ascites. The site of anastomosis of the blood vessel was occluded and buried in the recurrent tumor. Many collaterals were noticed.

Case of resection of portal vein with transplantation of polyethylene tube which is inserted and tied on both edges with 70% alcohol preserved vein homograft

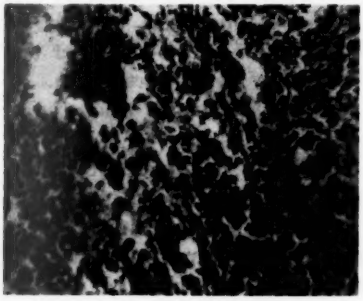
Case 1. Arai. 62 years old. Woman. Operative diagnosis: carcinoma of the bile duct.

The carcinoma occurred at the bile duct and infiltrated into the head of pancreas and gallbladder. The portal vein was also surrounded with intensive infiltration. Portal circulation was occluded for 40 minutes and the pathologic vein with carcinomatous invasion was resected 3.5 cm. long. Polyethylene tube inserted and tied on both edges with 70% alcohol preserved vein homograft (5 cm. long) was transplanted into the defect by non-suture method (Table II, 4). Though remarkable edema was noticed in the intestine, it disappeared at the end of operation. In spite of fall of blood pressure to 30 mm. Hg. during the operation, it returned to 110 mm. Hg. at the end of operation. The patient died 27 hours later by shock. At autopsy, no thrombus was found in the graft with good patency. Edema was not noticed in the intestine. The common hepatic artery, however, was found to be cut between ligatures. Pre- and postoperative microscopic pictures of the liver are shown in Fig. I. It is evident that the post-operative histology shows devastating picture compared to the preopertive one.

Fig. 1. Pre- and postoperative microscopic changes of liver. (Transplantation of portal vein and ligation of common hepatic artery)



Preoperative microscopic picture



Postoperative microscopic picture

Comment and Summary

Many experimental studies[1)-3)] on ligation of portal vein has been

reported. The acute fatal cause is attributed to shock. Child,[4] however, believed that the failure in dogs did not necessarily mean failure in human beings. Using Macaca monkeys, he obtained excellent results. He further reported two clinical cases without death by total ligation of the portal vein. Concluding from his experimental and clinical experiences, he demonstrated the possibility of ligation of the portal vein. In the literature, ligation of portal vein proximal to superior pancreaticoduodenal vein with survival has been reported.[5),6)] These cases have been done for therapeutic measures on the injured vessel. In cases of pancreaticoduodenectomy, however, superior pancreaticoduodenal vein is resected and ligated which prevents the establishment of collateral circulation toward the liver. Therefore, its ligation cannot be expected to be successful in human beings. All reported cases[7),8)] except Child's case with ligation of the portal vein during pancreatectomy died within few hours. The author's case also died within 4 hours after ligation. It is emphasized that ligation of portal vein should be avoided. According to the successful ligation of the portal vein by secondary or multiple operation,[9)] if abundant collaterals detouring the liver can be established, primary ligation may be possible. When carcinomatous infiltration extends to the portal vein, as have been recommended by Babcock,[10)] & Moore, resection of the infiltration to the blood vessel with lateral suture of the defect, end-to -end anastomosis or transplantation of the portal vein in order to send blood as much as possible to the liver may be useful. In several other cases not reported in this communication, slight stenosis after suturing the small defect in the portal vein did not show any complications. The reported 2 cases are one with stenosis of the portal vein and the other with symptoms of occlusion of the portal vein, respectively. Both cases teach us that careful anastomosis is absolutely required under such conditions.

Moore described various methods for resection of the portal vein. In case of large resection of the vessel, end to end anastomosis is very difficult. Anastomosis of portal vein with inferior vena cava cannot be applied frequently, because superior mesentric vein is often resected on the intestinal side. When porta-caval shunt is performed in normal liver, it is said that fatty necrosis of the liver,[11)] atrophy of hepatic cells[12)] and temporary lowering of liver function[13)] occurrs. Consequently, permanent cure of the body cannot be obtained by such anastomosis. For this reason, we haven't applied it in a single case.

Though anastomosis between superior mesenteric vein and splenic vein occludes frequently in experimental anaimals, as recommended by Ripstein,[14)] it may be used safely when healthy vessels are available. In the IIIrd case described in the present report shows its successful use which

is already reported by Katsura and Suzuki[15] from this department.

It may be easily understood that different method should be used in different cases. The most idealistic method, however, must be the replacement of the portal vein with grafts in case of pancreatectomy including the vessel lying directly behind it. Generally speaking, anastomosis and transplantation of vein is difficult and the results are unsatisfactory. The patency of portal vein after transplantation with grafts cannot be expected permanently as has been shown in chapter 1. These cases, however, survived the acute stage of operation and survived. This result was applied in the fourth case with a prothese inserted with human vein graft preserved in 70% alcohol by non-suture method. In spite of the accidental ligation of common hepatic artery, the portal circulation was maintained for 27 hours. This technique might serve as a hopeful method in the future.

All in all, management of portal vein in pancreatectomy is still accompanied with difficulty. One way to solve the problem is to diagnose and operate the tumor in an early stage when the growth is still confined to the pancreas and has no metastatic lesions on the surrounding tissues.

Conclusion

1. Of 31 cases which required pancreaticoduodenectomy, 27 showed adhesions and carcinomatous infiltration to the portal vein. 13 cases of primary carcinoma of the head of pancreas showed adhesions or infiltrations to the portal vein.

2. As soon as adhesion and infiltration to the portal vein is discovered, it is better to operate from the pancreatic side after transecting the pancreas toward the duodenum instead of beginning from the duodenum, to handle the blood vessels.

3. A case with ligation of the portal vein died 4 hours later after the accident.

4. Though a case with anastomosis between superior mesenteric vein and splenic vein was successful, the patient died with recurrence of the cancer and peritonitis carcinomatosa several months later. The anastomosis was compressed and obstructed by growth of the lesion.

5. Transplantation of polyethylene tube inserted and tied on both edges with 70% alcohol preserved vein homografts was performed by non-suture technique. At autopsy, in spite of ligation of common hepatic artery, the graft was patent without obvious thrombus for 27 hours till death.

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The Effect of a Small Dose of Roentgen Rays upon the Human Body as Revealed by the Method of Electric Flicker

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Introduction

It was reported by Brandes[1] (1896) that a sensation of light was evoked by Roentgen rays, and this finding was confirmed by a number of subsequent investigators. Himstedt and Nagel[2] found that in response to an irradiation by Roentgen rays a retinal action current very similar to that elicited by photic stimulation appeared. Lipetz[3] showed that the threshold energy of Roentgen rays was close to that of visible light for dark-adapted frog eyes. For dark-adapted human eyes it is said that a dose of Roentgen rays from 0.5 to 1.4 r per minute for an area of 1 mm.^{2} of the retina is sufficient to evoke Roentgen phosphenes.[4] It does not seem likely that the fluorescence of the lens and the retina induced by Roentgen rays should be responsible for Roentgen phosphenes.[5][6] Newell and Borley[4] showed that the threshold for Roentgen phosphenes varied with the process of dark adaptation in the same manner as the threshold for visible light, and this was confirmed by Lipetz on frog retinae, who measured the threshold, taking spike potentials from ganglion cells of the retina as an index. He further demonstrated that visual purple was bleached by Roentgen rays.

All the data stated above indicate that Roentgen rays act upon the retina like visible light to cause a sensation of light.

Recently Tarusawa *et al.*[7][8][9] found that a sensation of light caused by electrical stimulation of the eye was affected by Roentgen rays; they irradiated various parts of the human body with high doses of Roentgen rays for the purpose of Roentgen therapy, and found that the threshold voltage to produce a light sensation increased following an irradiation by Roentgen rays. The effect was found conspicuous even when regions

remote from the eyes such as legs were irradiated, and some serological effects could be observed following the irradiation. So they interpreted the increase in electrical threshold of the eye as a secondary effect of the general serological effect.

As the local effect of scattered Roentgen rays is not ruled out in their experiments, it is a question whether their interpretation is correct or not. We attempted, therefore, to determine the threshold intensity of Roentgen rays to produce a noticeable increase of electrical threshold of the eye, and to measure scattering precisely during irradiation of any other parts of the body.

Experimental

Method

Historical notes on the method of electric flicker

It has long been known that a sensation of light is elicited by electrical stimulation of the human eye, and this sensation is called "an electrical phosphene." Motokawa and Iwama[10] found that the electrical threshold for phosphenes was very sentitive to oxygen lack. In this experiment they used rectangular pulses of 20 cps., and measured the threshold S_1 for appearance of phosphene by increasing the stimulating current at a certain constant rate from zero to the threshold, and further the threshold S_2 for disappearance of phosphene by decreasing the current from a high level to the threshold at the same rate as above. The average of the two kinds of threshold, S_1 and S_2, did not show any change when the observer was subjected to a low atmospheric pressure corresponding to high altitude of about 3 km., but it was found that the effect of such a slight decrease of atmospheric pressure manifested itself in the difference between S_1 and S_2. On exposure to oxygen lack of much higher degrees the difference ΔS and the average of S_1 and S_2, increased almost in parallel so that there was no reason to prefer ΔS to the average as an indicator of oxygen lack.

Motokawa and Suzuki[11] applied the same method for measuring fatigue, based on the fact that ΔS is very sensitive to oxygen lack of a slight degree. Mita *et al.*[12] changed the rate of increase or decrease of the stimulating current to study the effect of the rate on ΔS, and found that ΔS was remarkably influenced by the reaction time of the observer when the rate was too high; S_1 became greater and S_2 smaller so that ΔS increased with the rate. This factor, however, dropped out when the rate was too low, and in turn such factors as electrical sensitivity and its change or adaptation were found to play a more important role. At an intermediate rate both time factor and sensitivity factor were effective at the same time, and it was at such intermediate rates that ΔS could serve as a sensitive indicator for oxygen lack or fatigue. It is conceivable that both of them

together can give more information about oxygen lack or fatigue than does either of them alone, especially when the degree of oxygen lack or fatigue is slight.

Suzuki[13] applied this method for measuring fatigue in a variety of professional tasks and exercises. Tsukahara[14] used an electronic low frequency oscillator instead of Motokawa's original apparatus, because the effect of the skin resistance on the stimulating current was not eliminated in the original apparatus. Mita and his co-workers studied the effect of illumination upon ΔS, which was found to increase both at extremely high and low illuminations. They determined a range of illumination adequate for ΔS-measurements; it was a range between 1 and 100 lux within which ΔS showed only slight dependence on the illumination. Nakamura *et al.*[15][16] studied the correlation between ΔS and impairment of pulmonal function in silicosis, and found that increases of ΔS caused by a load could be a reliable indicator of impaired function of the lung; the correlation coefficient between ΔS and vital capacity was found very high, whereas no parallelism was found between ΔS or vital capacity and Roentgenograms of the lung.

The apparatus used in the present experiment

Toadenpa & Co. in Tokyo undertook the construction of a convenient, practical apparatus after Motokawa's suggestion. The apparatus consists of a generator of sinusoidal alternating current of 20 cps. and a potential divider. The frequency of the current was fixed at 20 cps., because at this frequency the electrical threshold of the eye is lowest, and no advantage has ever been brought about by the use of any other frequencies. The output voltage from the potential divider can be increased or decreased automatically at a definite uniform rate by means of a device to be mentioned later. A high resistance of 120 kohms inserted into the circuit of the head of the subject serves to eliminate the effect of the skin resistance. The greatest output of this apparatus is 300 μA in view of the fact that threshold current is usually below 100 μA. When a synchronous motor driving the potential divider is switched in, the current increases from zero at the constant rate of 12 μA per second, which was determined based upon Mita's experiment mentioned above. On perceiving the appearance of electric flicker, the observer presses a key to stop the motor. Thus the experimenter can read the threshold current, S_1. When the observer releases the key, the current increases further so as to cause more distinct electric flicker. When the current has increased by a certain definite amount above the threshold for appearance, a clutch works automatically to reverse the direction of excursion of the potentiometer, and the current decreases at the same uniform rate till the observer presses the key on perceiving the disappearance of electric flicker. The experimenter reads

S_2, and after that, the observer releases the key again to reduce the current towards zero. This operation is repeated, and some pairs of S_1 and S_2 are determined successively. The device is represented schematically in Fig. 1.

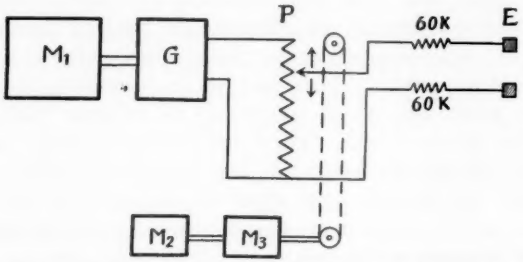

Fig. 1. Apparatus for measuring electric flicker. E stimulating electrodes. G generator of alternating current of 20 cps. M_1, M_2, M_3 synchronous motors. P potentiometr whose electric contact is moved up and down as indicated by arrows by means of M_2 and M_3.

As stimulating electrodes silver plates of 2×2 cm.^{2} in size were used which were held in place on symmetrical parts of the skin of the outer corner of the eyes, the contact between the skin and the electrode being secured with electrode paste.

Conditions of illumination were as follows: A plate of opaque glass 20×20 cm.^{2} in size illuminated uniformly from behind was placed at a distance of 25 cm. in front of the eyes of the observer, who fixated the center of the visual field during measurements. The illumination was 10 lux. Conditions of Roentgen irradiation were as follows: Secondary electric voltage 70 k.V. Electric current 3 mA. Filter used was an alminum plate 1.0 mm. in thickness. Area of irradiation 10×10 cm.^{2} Distance from the focus to the skin 30 cm.

Evaluation of data

Concerning the electrical sensitivity expressed in terms of S_1 or S_2 there are great individual variations so that it is difficult to set up any norm concerning these values. The same is true of ΔS. Therefore it is important to make comparison of data obtained from one and the same subject before and after any treatment whose effect is to be investigated, or else statistical evaluation is always necessary. In about 80 per cent of our subject, about 30 in number, values of ΔS remained within 10 and 30 μA, and standard deviation of 10 measurements was 3 to 6 μA. Without load a complete parallelism is found between S_1 and ΔS, and they can vary parallel to each other under some stress. Scattering of values is usually greater at the start than in later stages. Therefore it is advisable

to discard some data obtained in the beginning.

Results

Values of electric flicker or ΔS were measured before and after a brief irradiation of the eyes with Roentgen rays of 0.5 r. The data obtained are shown in Fig. 2, in which each value of ΔS obtained from any two successive measurements of S_1 and S_2 is marked by a cross and plotted against time. The moment of irradiation is taken as zero. The average

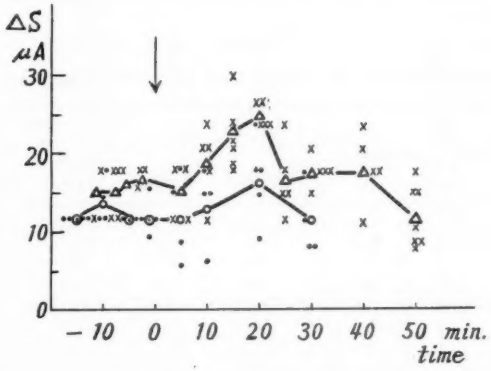

Fig. 2. Changes of flicker values, ΔS, following Roentgen irradiation of 0.5 r. Arrow indicates irradiation. Curve connecting open circles was obtained from a control experiment in which the eyes were covered with a lead plate.

of a few or several values of ΔS determined successively is represented by a triangle in the figure. One may get an idea of fluctuation of ΔS from scattering of crosses, and find an effect of irradiation in a temporary increase in the average values of ΔS following the irradiation. In order to assure that this effect was really due to Roentgen rays, a control experiment was carried out as follows: The eyes were covered with a lead plate 10×10 cm.^{2} in size and 2.5 mm. in thickness, and then the same procedure was performed as in the above experiment. The data obtained are represented by dots in Fig. 2, the average values of consecutive ΔS being represented by open circles. As can be seen in this figure, the increase of ΔS following the irradiation is not so conspicuous as in the previous case; the slight trend to increase in the present case may not be statistically significant in view of the considerable scattering of individual values of ΔS. As a matter of fact, the difference between the ΔS-values before and after the irradiation was shown to be statistically significant in the experiment without the lead cover over the eyes, but insignificant

in the control experiment. These experiments indicate clearly that Roentgen rays were the direct cause of the increase of ΔS. It is to be noted that this effect is not any secondary effect of visual excitation, because no sensation of light was evoked by Roentgen rays of the dose used in the above experiment. Next, the effect of Roentgen irradiation of the other parts of the body was investigated. The chest and the abdominal regions of 3 subjects were exposed to Roentgen rays of 0.5, 1.0 and 5.0 r, and measurements of ΔS were carried out as above. The data obtained are summrized in Table I. It is apparent from this table that no such

TABLE I

Changes of ΔS in μA before and after Roentgen Irradiation

Irradiated region	Dose	Subject	Flicker values				
			Before	After 5 min.	After 10 min.	After 20 min.	After 30 min.
Abdominal region	0.5 r	J. U.	15.0	15.6	16.2	15.6	16.8
		S. K.	24.3	17.8	25.2	21.9	22.2
		M. K.	28.2	28.4	31.8	28.2	29.4
	1.0 r	J. U.	15.6	16.8	16.5	13.8	13.8
		S. K.	18.6	18.6	18.6	24.0	21.0
		M. K.	26.4	26.4	27.0	27.0	27.6
	5.0 r	J. U.	13.2	13.5	12.0	12.6	13.8
		S. K.	22.2	22.2	24.3	28.8	23.4
		M. K.	21.6	24.3	24.0	25.8	21.0
Chest region	0.5 r	J. U.	13.2	14.4	12.6	13.2	13.8
		S. K.	19.2	17.4	22.2	18.0	19.8
		M. K.	16.5	16.5	15.0	16.2	15.6
	1.0 r	J. U.	9.6	10.2	10.8	10.2	12.0
		S. K.	13.8	17.4	15.0	13.8	13.2
		M. K.	22.8	22.8	26.4	25.8	27.0
	5.0 r	J. U.	11.4	13.2	12.6	10.8	9.0
		S. K.	14.4	15.6	13.2	17.4	17.4
		M. K.	15.9	17.4	15.6	18.0	19.8

remarkable effect as observed in the direct irradiation of the eyes was caused even with greater doses such as 1.0 and 5.0 r. In some subject, however, a slight increase of ΔS could be noticed following an irradiation with 5.0 r. This effect may, however, be accounted for in terms of the effect of scattered Roentgen rays, because some degree of blackening happened in a Roentgen film placed upon the eyes when the dose of Roentgen rays irradiating the chest and the abdominal parts was as high as 5.0 r.

In what follows some experiments were performed to determine the threshold dose of Roentgen rays to produce a noticeable increase of ΔS when the eyes were irradiated directly. The data are shown in Table II. The difference between values obtained before and after each irradi-

TABLE II

Determination of Threshold Dose of Roentgen Rays

Subject	Dose in r	Flicker values, ΔS in μA						
		Minutes after irradiation						
		Before	5	10	15	20	25	30
J. U.	0.05	15.0	14.7	11.4	13.5			
	0.1	16.2	14.1	14.7	18.6			
	0.2	18.6	24.9	16.8	16.8			
	0.3	15.9	24.3	31.2	32.1			
S. K.	0.1	21.3	19.5	24.0				
	0.2	21.6	18.0	21.0		16.8		
	0.3	18.0	19.8	21.0		18.0		
	0.4	19.8	22.5	30.6		21.9		
M. K.	0.1	15.6	12.0	18.0	16.8	15.0		
	0.2	13.2	15.0	15.6		15.0		
	0.3	17.1	17.4	17.1		20.2		
	0.4	13.2	22.8	27.6		21.0		15.6
K. S.	0.05	11.7	6.3	11.1	10.8			
	0.1	3.9	12.0	13.8	5.7	4.8	3.0	3.0

ation was subjected to a statistical test, and it was found that the minimal dose required to produce a noticeable increase of ΔS lay between 0.1 and 0.4 r; the threshold was 0.3 r. in subject J. U., 0.4 in S. K., 0.4 in M. K., and 0.1 in K. S. As can be seen in Fig. 2, the effect of Roentgen rays becomes manifest with a latency of some minutes, attains a maximum in about 20 minutes, and disappears in 30 to 60 minutes. In the range between 0.1 and 5.0 r. the time to the crest of the effect depends little on the dose of Roentgen rays, but the recovery time seems to become longer as the dose increases.

DISCUSSION

As has been demonstrated by the experiments of the previous investigators mentioned above, there is little doubt that Roentgen rays act upon the receptor system of the eye and cause a sensation of light. Although it is certain that the present phenomenon is concerned with a direct action of Roentgen rays upon the retina, but not with such a general effect as has been assumed by Tarusawa *et al.*, the increase of ΔS following an irradiation with Roentgen rays has no direct connection with the visual effect of the Roentgen rays. It is because no sensation of light was caused by so small doses of Roentgen rays as used in our experiments and in a light-adapted state of the retina, and because the threshold energy of Roentgen rays is even lower for our phenomenon than for Roentgen phosphenes of the dark-adapted retina. As has been shown above it

takes about 30 to 60 minutes for the increased ΔS to return to the initial level, and this long recovery time could not be accounted for in terms of the after-effect of visual excitation, especially under light-adapted conditions of the eye; it is known that the recovery of the photopic visual system from preceding excitation is usually completed within about 10 minutes. The mode of action of Roentgen rays should be regarded as different from the mode of action of visible light, so far as the present phenomenon is concerned.

There are grounds to assume that the site of electrical stimulation of the eye would be neuronal elements of the retina, but not the receptor cells themselves. The electrical sensitivity of the retina takes a different course during dark adaptation from the photosensitivity. The retina shows after a brief illumination supernormal sensitivity to electrical stimulation, as has been shown by Motokawa, but no such supernormal sensitivity to photic stimulation. If it is assumed that the neuronal elements of the light-adapted retina are more sensitive to Roentgen rays than are the receptor cells, the present phenomenon may be regarded as an event in the nervous net work of the retina; the depressed electrical excitability of the neuronal elements following a Roentgen irradiation is not a refractory state, because it is not preceded by excitation. It must refer to some deteriorating action of Roentgen rays, although our phenomenon is caused by a very small amount of Roentgen rays and only temporary. This phenomenon would be valuable for investigation of biological effects of Roentgen rays and prevention of intoxication, because the effective dose of Roentgen rays is much lower for this phenomenon than for any others, and the method of measurement is practical enough to find wide application of it.

Summary

The method of electric flicker which is serviceable for measuring the effect of oxygen lack and fatigue was shown to be useful for detection of the effect of a small dose of Roentgen rays, too.

1. The threshold value of Roentgen rays to produce a noticeable increase of the electric flicker value was within a range from 0.1 to 0.4 r.
2. The effect of a Roentgen irradiation appeared with a latency of a few minutes, and attained a maximum in about 20–30 minutes to disappear in about 50 minutes.
3. As no sensation of light was elicited by such a small dose of Roentgen rays in a light-adapted state of the retina, the phenomenon under consideration is not a secondary effect of visual excitation.
4. When any other parts of the body were exposed to Roentgen rays of higher doses, a very slight effect, if any, could be observed, and

this effect could be accounted for in terms of the direct effect of scattered Roentgen rays upon the eyes.

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Retinal Responses to Intermittent Light of Subfusional Frequencies

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Introduction

The phenomenon of flicker has hitherto been studied by various methods, for example, by the method of CFF, by ERG, by EEG and by other psychological methods.

Bartley[1] reported that at some subfusional pulse frequencies the brightness of the pulses appears greater than the brightness of steady illumination of the same physical intensity. This enhancement was found maximal in the neighborhood of 10 pulses per second. This rate approximately coincides with the alpha rhythm, and this coincidence seemed to him not accidental because both photic stimulation of the retina and electric stimulation of the optic nerve were found maximally effective in producing responses at the optic cortex when delivered at the rate of the alpha rhythm. This finding seems to suggest that Bartley's effect occurs at the visual cortex, but cannot rule out the possibility that this phenomenon may occur at the retinal level.

The effect of light on the retina manifests itself in increases in electrical excitablity of the eye following an illumination (Motokawa *et al.*[2]). A percentage increase which is denoted by ζ varies in proportion to the logarithm of the light intensity over a wide range of intensities just as does CFF (Suzuki and Ooba[3]).

If Bartley's effect or enhancement of excitation at subfusional rates of flicker occurs at the retinal level, it is expected that a corresponding change should occur in Motokawa's effect. This notion was justified in the present investigation.

Experimental

Method

The method for determination of electrical excitablity of the eye is

described in detail in previous papers,[4)5)] so that only points modified in the present experiment will be described. Flickering light was produced by means of a rotating sector of light-dark ratio 1:1. The rate of flicker was measured by a well-calibrated electric tachometer attached to the apparatus.

An opaque glass disc of 1 cm. in diameter was illuminated from behind by intermittent white or spectral light and was viewed from a distance of 40 cm. so that the visual angle subtended by the disc at the eye was 1°26′. The spectrum used was an equal-energy spectrum. The light intensity was controlled by a neutral wedge and by changing the distance from the light sources. Experiments were carried out at the fovea and in the periphery 50° from the fovea.

After a preliminary dark adaptation of about 20 minutes the electrical excitability of the left eye was measured without pre illumination and after exposure of the eye to the illuminated disc mentioned above for 2 seconds, if not otherwise stated. The test electric stimulus, a rectangular pulse of 0.1 second was delivered at different times after termination of the photic stimulus according to the wave-length of the pre-illuminating light.

Results

The ζ -frequency curves obtained at the fovea for white lights of 525, 52.5, 5.25 and 0.52 lux are illustrated in Fig. 1. In this figure, percentrage increases in electrical excitablity of the eye over resting level (ζ) are plotted as ordinates against frequencies of flickering white light used for pre-illumination as abscissas. The CFF for each intensity of white light is indicated with a vertical arrow. When such relatively high intensities as 525, 52.5 and 5.25 lux were used, the curves indicated two elevations having prominent maxima at about 7–10 and 20 cps. respectively, but at such a lower intensity as 0.52 lux a single maximum at about 15 cps. appeared.

Next, ζ-frequency curves were obtained from other three subjects (Katayama, Aizawa and Kobata) under the same experimental conditions as stated above. The light intensity used was 5.25 lux. The data obtained are shown in Fig. 2. As can be seen in this figure, all curves show maxima at about 10 and 20 cps., although some individual differences can be seen about magnitudes and shape of the curves. Their CFF were found 40, 35 and 35 cps. respectively. These results are almost identical with those shown in Fig. 1.

Setting aside the maximum at about 20 cps., the phenomenon observed above coincides with Bartley's observation that the brightness of flickering light is maximally enhanced at about 10 cps., because enhance-

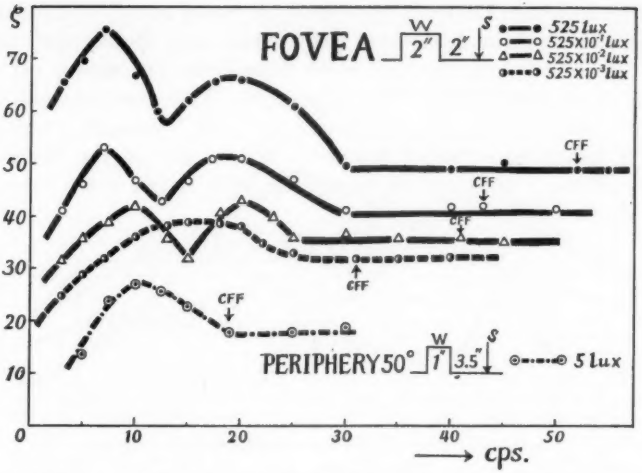

Fig. 1. Effects of subfusional intermittent lights of various intensities as a function of frequencies of flicker. Ordinates: ζ or percentage increases of electrical excitability following exposure to intermittent light. Abscissas: frequencies of flicker in cps. Temporal sequences of procedure are shown in inset where W and S denote flickering white light and electric stimulus of 0.1 sec. in duration respectively. Curve at bottom refers to rod-process.

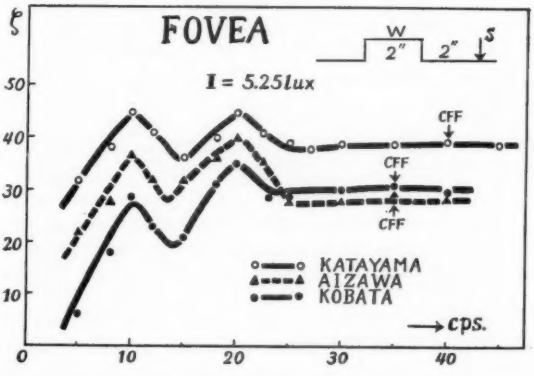

Fig. 2. ζ-frequency curves obtained from 3 subjects.

ment of electrical excitability means that the effect of the pre-illuminating light upon the retina was stronger at this rate than the other rates of flicker.

Similar measurements were carried out with red (650 mμ), green

(530 mμ) and blue (470 mμ) lights from an equal energy spectrum. The results obtained from 2 subjects (Motokawa and Ooba) are shown in Fig. 3. In this figure, the curves for Motokawa are shown on the left side and those for Ooba on the right side. The time interval between the photic and the electric stimuli was 1, 2 and 3 seconds for red, green and blue lights respectively, because the maxima of the electrical excitability in the ζ-time curves lie usually at 1, 2 and 3 sec. respectively

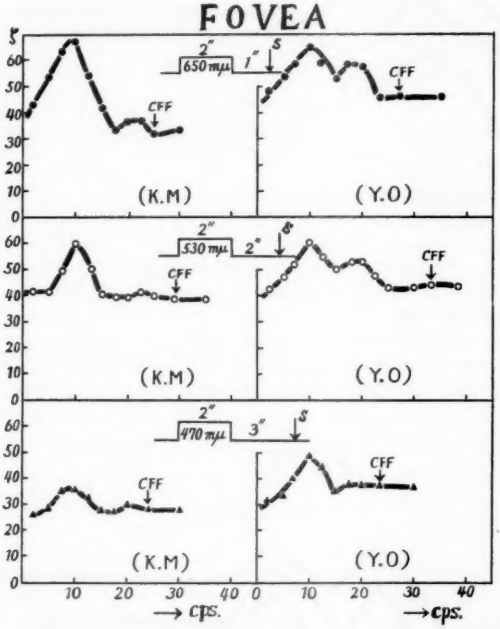

Fig. 3. ζ-frequency curves obtained from 2 subjects with various spectral lights at fovea. Curves marked by solid circles, empty circles and solid triangles were obtained with spectral lights of 650, 530 and 470 mμ respectively.

when red, green and blue lights are used for pre-illumination.[4] As can be seen in this figure, all curves show their maxima at about 10 and 20 cps., and the elevation at about 10 cps. is most conspicuous for red light and least prominent for blue light.

In the following experiment, spectral response curves were obtained by the method described in the previous paper.[4] The curves for stationary lights R, G and B are marked by empty circles, empty triangles, and empty squares in Fig. 4. These three curves, R, G and B refer to the red, the

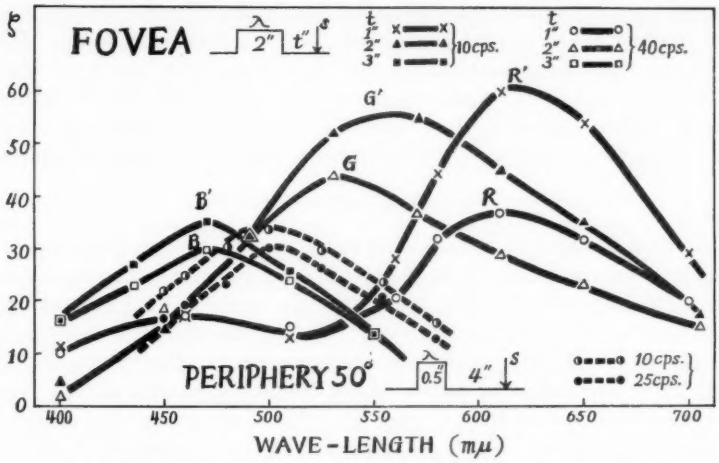

Fig. 4. Response curves to steady spectral lights and to those flickering at 10 cps. R, G and B refer to responses of red, green and glue receptors to steady lights, and R′, G′ and B′ represent corresponding responses to flickering lights. Scotopic response curves obtained at 50° from fovea are represented by broken curve marked by solid circles for stationary lights and by that marked by semicircles for flickering lights.

green and the blue receptors, respectively. When flickering spectral lights of 10 cps. were used instead of stationary lights, response curves R′ (marked by crosses), G′ (marked by solid triangles) and B′ (marked by solid squares) were obtained. It is apparent from these results that the response curves for flickering lights are always higher than those for corresponding stationary lights. Enhancement brought about by flicker is found very marked, especially at the parts of longer wave-lengths.

The experimental results stated above are with respect to the cone-responses, and so in the following experiment the rod- process was measured at periphery 50°, in order to study the effect of flicker upon the rod-response. The ζ-value was measured at 3.5 sec. after exposure to flickering light for 1 second, because the rod-process shows its maximum 4.5 sec. after the onset of light, as was shown by Motokawa and Ebe[6] and by Oikawa.[7] The rod-response as a function of flicker rates is illustrated at the bottom in Fig. 1. As can be seen in this figure, the curve for the rod-process shows a single maximum at about 10 cps. and no second maximum as do the curves for the cone-processes.

Next, spectral response curves of the rod-process were obtained with steady spectral lights and with those flickering at a rate of 10 cps. at periphery 50°. The data are shown by broken curves in Fig. 4. The

curve marked by solid circles refers to the steady illumination, while the curve marked by semi-solid circles refers to the flickering lights. These two curves have their maxima at the same part of the spectrum (about 500 mμ) as the scotopic visibility curve, and the curve for the flickering lights is higher only slightly. This finding indicates that the effect of flicker is much weaker upon the scotopic system than upon the photopic.

Discussion

As to the effect of intermittent visual stimulation the interest of investigators has centered about the CFF, and researches on the effect of subfusional intermittent light are extremely scarce. This is because investigation of a transitional phenomenon is generally more difficult than a steady one. Bartley made his observers match the brightness produced by a series of light pulses to the brightness produced by a steady light, and found that the average physical intensity of the subfusional intermittent light was not so great as that of the continuous illumination equally effective in brightness. Based upon the fact that when the pulsation rate was in the neighborhood of 10 cps. enhancement was at its maximum, Bartley surmised that this effect might be correlated with the alpha rhythm of the brain.

The present investigation has shown that a similar enhancement occurs at about the same pulsation rate with regard to the electrical excitability of the eye following an illumination with intermittent light. Therefore, the present phenomenon seems to represent a physiological correlate of Bartley's effect. Both of these effects suggest that visual excitation is greater in the transition period than in the stationary state.

The evidence of this can be seen also in the discharge pattern of the optic nerve as was shown by Adrian[8] and by Hartline.[9] On the onset of a continuous illumination the impulse frequency rises rapidly, reaches a maximum, and declines slowly to reach a steady level which continues so long as the illumination lasts. Broca and Sulzer[10] studied the brightness of a single flash of light in comparison of that of a steady light, and found that the brightness of a flash of a certain intermediate duration was much greater than that of the steady light of the same physical intensity. The time from the onset of illumination to the maximum of brightness seemed to depend upon the intensity and the wave-length of the light used. So far as Bartley's effect is concerned, the dark-light ratio had no effect upon the optimal frequency of the pulses, and in our phenomenon it did not seem that the intensity had any effect upon the optimal pulsation rate, unless it was too low.

Although Bartley emphasizes the correlation between his effect and the alpha rhythm, based upon the similarity of the optimal frequency

for his effect to the alpha rhythm, some reservation seems to be necessary for this assumption. The reasons for this reservation are as follows:

1) The optimal frequency for Bartley's effect is, on an average, little lower than the alpha rhythm, and the same is true of our phenomenon.

2) A second enhancement is found at about 20 cps. in our phenomenon. This enhancement is usually not so great as that found at about 10 cps., but may be measured with greater ease and certainly in some subjects. It is apparent that this second optimal frequency has no connection with the alpha rhythm. We have yet no evidence that there is any steady rhythm of about 20 cps. in the human brain. On the contrary it is an established fact that the human eye shows the lowest threshold for electrical pulses or alternating currents of about 20 cps.[11)13)] Therefore, it seems more probable that photic activation caused by intermittent stimulation occurs at the retinal level.

According to Brazier[14)] and Van Buskirk *et al.*,[15)] intermittent red light is more effective than other colored lights to evoke seisure waves in epileptic patients, and this fact is in line with our observation that intermittent spectral lights of longer wave-lengths are most effective to cause enhancement of electrical excitability of the eye.

Summary

The effect of intermittent lights of subfusional frequencies was investigated by the method of electrostimulation of the eye. The light-dark ratio of flickering light was always 1:1.

1. Increases of electrical sensitivity following an illumination with intermittent white light were usually greater below the CFF than above it. When the electrical sensitivity after an illumination was plotted against the rate of flicker of the illuminating light, the curve showed two prominent maxima at about 10 and 20 cps.

2. The same effect could be seen when monochromatic lights of various wave-lengths were used instead of white light.

3. At the flicker rate of 10 cps., the degree of enhancement of electrical excitability was found highest for red light, intermediate for green light, and lowest for blue light.

4. The rod-process received no such conspicuous enhancement as the cone-processes.

5. The above-mentioned effect was correlated with the psychological phenomenon described by Bartley that a light flickering at a subfusional rate looks brighter than a fused one. Based upon the wave- length dependence of this phenomenon a retinal mechanism was suggested for photic activation of seisure waves with intermittent stimulation.

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Functional Determination of Rod Free Area of Human Retina

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Introduction

There are two kinds of receptors, rods and cones in the human retina. The rods play a main role in twilight vision, and the cones in daylight vision. Hitherto information about the function of the rods has been obtained from experiments in which light of such a low intensity was used that it scarcely excited the cones. On the other hand, researches on the function of the cones[7)-10)] have been performed mainly at the rod free area of the retina in order to avoid any interaction between cones and rods. Therefore it is important to know how wide the rod free area is. With regard to the rod free area many papers[1)-3)] have been published: Koster[4)] concludes that the rod free area measures about 800 μ in diameter, subtending a visual angle of 3°3′. According to Oesterberg, rods do not appear at all until at 130 μ from the centre of the fovea (260 μ in diameter, 53.2′ in visual angle). However, the region of the fovea which Polyak[6)] regards as entirely free from rods is about 500 μ in diameter and subtends a visual angle of 1°40′.

All the results described above have been obtained by histological observation. They, however, show remarkable differences from one another in regard to the size of the rod free area. Therefore we tried a further investigation with a quite different method of approach based on the different characteristics of cones and rods with regard to electrical sensitivity after conditioning illumination. As stated above, it is necessary to restrict the intensity of illumination to below the threshold of the cones for studying the function of the rods independently of that of the cones in retinal regions where the two kinds of receptors co-exist. Motokawa and his associates[11)12)] have provided a means by which a rod process and a cone process can clearly be separated from each other. This method[13)] consists in tracing the time course of the electrical sensi-

tivity of the eye after exposure to a brief illumination. Since this method is based on differences in the time factor of visual processes, but not in their intensity factor, it may be applied to any experiment without such limitation about the intensity of illumination as stated above. We explored the spatial distribution of the rod function in the fovea by means of Motokawa's method and compared it with the histological results obtained so far. According to the Ladd-Franklin's theory on color vision a smaller rod free area is to be expected in color-blind subjects because color differentiation is not so complete in their foveae as in those of normals. So we explored the function of the rods in the foveae of partially color-blind subjects by the same method and studied the distribution thereof in comparison with the distribution determined in normal subjects.

Experimental

Method

The method has been fully described elsewhere,[12)13)] so that the details are omitted here. A preliminary dark adaptation of about 40 minutes is allowed before making any electrical and photic stimulation. Following an illumination the electrical sensitivity of the dark-adapted eye is measured with electrical phosphenes as an index. The sensitivity increases above normal after an initial brief depression, reaches a maximum in a few seconds and decreases gradually to the resting level. For convenience, percentage increases in electrical sensitivity over its resting level are denoted by ζ. The time course of this change depends on the sort of the receptors excited. The time courses for red, yellow, green and blue receptors attain a maximum at 1, 1.5, 2 and 3 seconds respectively after termination of a photic stimulus. The time from the end of illumination to the maximum will be called a "crest time." The time course for the rod process is known[11)12)] to reach its maximum at 4.5 seconds from the onset, instead of from the end of illumination. This is because the rod process is mediated by pure on-elements, as suggested by Granit[16)] and confirmed by Motokawa and Ebe.[11)] As the duration of illumination is made 0.5 seconds in our all experiments, the crest time for the rod process is 4.0 seconds from the end of illumination. The rod process is slower than the blue process which is slowest of all the cone processes. The difference between the crest time for the rod process and that for the blue process is 1 second.

In researches on the rod process, we used blue-green light of 510 mμ as illuminating light, because it was most effective to excite the rods.[12)] Of course this spectral light can excite the blue receptor together. Therefore ζ values measured at 4 seconds from the end of the photic stimulus

must be checked whether they refer to the rod process or to the blue process. For this purpose, we have only to trace a ζ-time curve. If the crest time of the curve is 3 seconds, the curve refers to the blue process, and if it is 4 seconds, it is the rod process.

Color vision test

Tests of color vision were made with Ishihara's test and Nagel's anomaloscope. The former was used for rough screening. The final decision of the type of color defect was made on the basis of a systematic test with the anomaloscope.

Errors of measurements

As the excitability of the resting eye can fluctuate the resting threshold was measured from time to time in every series of experiment to be sure of its stability. The fluctuation usually remained within 10% within a session. Otherwise data were discarded. When one series of experiment could not be furnished in a session, it was continued in another session, because good reproducibility of data allowed it. ζ values obtained under one and same experimental condition usually coincided with one another within the error of about 2. An example of experimental errors in a well trained subject is shown in Table I.

TABLE I

Experiment Showing the Degree of Accuracy of Measurement

Retinal position: 2° from center of fovea.
Area illuminated: 3 minutes of arc in visual angle.
Illuminating light: blue-green light (510 mμ).

Session	ζ values (rod)					Mean and standard deviation
I	5.8	5.2	5.8	5.4	5.9	5.52±0.296
	5.5	5.6	5.8	5	5.2	
II	5.8	5.6	5.1	6	5.3	5.51±0.250
	5.4	5.6	5.5	5.5	5.5	

Results

Experiment 1

The fovea of the normal subject was exposed to circular patches of various sizes illuminated by the spectral light of 510 mμ. The size of patches was varied from 8′ to 2° in diameter. The intensity of the spectral light was varied by means of a neutral wedge which was placed between the light source and the spectroscope. The relation between the magnitude of the rod process and the logarithm of the light intensity is represented graphically in Fig. 1. In this experiment two fixation lights were used so as to fixate the center of the circular patch half-way between them.

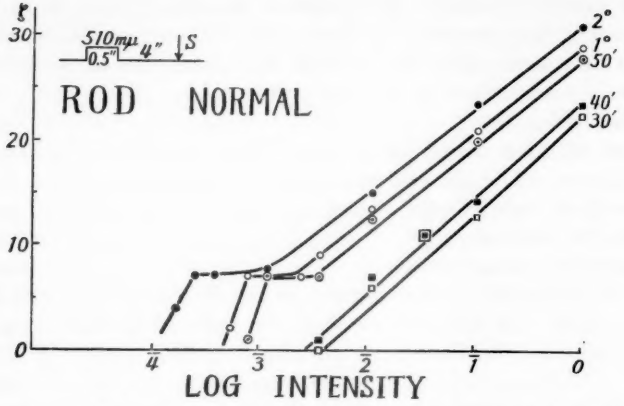

Fig. 1. Relation between magnitude of rod process and log intensity of illumination in normal subject. The curves were obtained from illuminating areas 30′, 40′, 50′, 1° and 2° in diameter. Ordinates: ζ or percentage increases in electrical sensitivity above resting level following illumination. Abscissas: Log intensity of illumination. The duration of light stimulus was 0.5 seconds. Temporal sequence of procedure is shown in inset where S denotes electrical test stimulus.

As can be seen in each curve, the magnitude of the rod process decreases in linear relation to the logarithm of the intensity as the intensity of the spectral light is reduced. However, when the size of the patch is greater, the magnitude of the rod process remains constant in a lower range of intensities. Such a range with constant ζ is lacking with patches of smaller areas. Moreover, this ζ constant intensity range agrees with the photochromatic interval in which subjects feel only brightness, but not color. Previously Oikawa[14)15)] reported that between the light- and color-thresholds the magnitude of the rod process remained constant irrespective of the intensity. This statement conforms to our results. The magnitude of the rod process (ζ) decreases with decreasing intensities, but below the color-threshold it remains almost constant over a certain intensity range and decreases again to disappear at the light-threshold.

On the other hand, with smaller patches there is no such intensity range in which ζ remains constant; ζ decreases merely in linear relation to the logarithm of intensities. This simpler type of relation between ζ and intensity will be called " type I " and the complicated type " type II."

Next, we measured ζ-time curves to make sure that the difference between these two types depends on whether the rods are contained in the measuring area or not.

The curves obtained are represented in Fig. 2 in which the upper set of curves refers to type II, and the lower to type I. The duration of illumination was 0.5 seconds as stated before. In the upper set (type II),

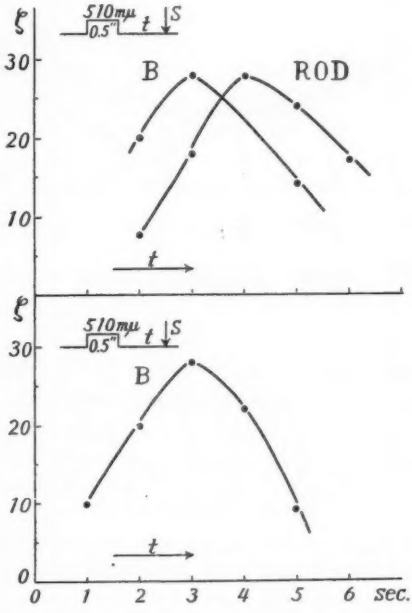

Fig. 2. Time course of electrical excitability after illumination. Abscissas: Time after end of illumination. Upper diagram refers to type II. Lower diagram to type I.

one can see in addition to the blue process the rod process which has a "crest time" at 4.0 seconds from the end of illumination, while in the lower set (type I) there is no rod process, but only the blue process which has a "crest time" at 3 seconds. Thus it has been confirmed that the rod process characterizes the type II. In other wards, the intensity range with constant ζ is characteristic of the rod process. This range is missing in type I because no rod exists in the retinal area illuminated by a patch of smaller size. Utilizing this criterion it is possible to determine the extent of the rod free area. The data illustrated in Fig. 1 show that the rods do not exist at all in the central area of the fovea 40′ in diameter in visual angle but begin to appear in the area of 50′ in visual angle.

Next, ζ-intensity curves which were obtained from color-blind subjects are illustrated in Fig. 3. As can be seen in this figure, the two types I

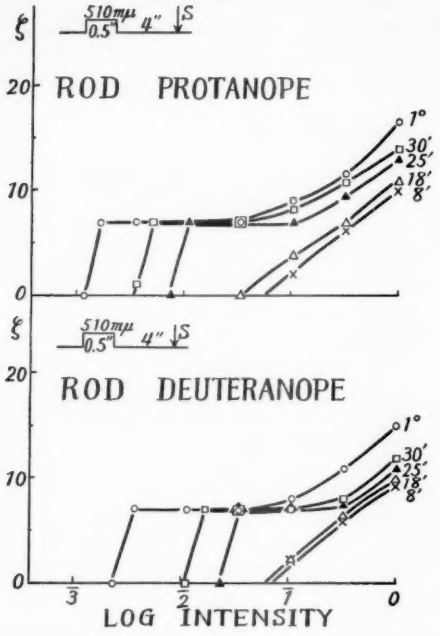

Fig. 3. Relations between magnitudes of rod process and log intensity of illumination in color-blind subjects. Explanation as in Fig. 1.

and II can be distinguished here again. From these curves, it can be said that the rods do not appear at all in the central area of 18′ in diameter and appear first in the area of 25′ in the color-blind subjects.

Experiment 2

Various points of the fovea were exposed to a target of 3′ in visual angle to obtain the relation between the exposed retinal position and the magnitude of the rod process. Measurements were carried out along the horizontal meridian. In Fig. 4 this relation is illustrated. The smaller the distance from the foveal centre is, the smaller becomes the magnitude of the rod process, and the rod response does not appear when the distance is reduced below a certain limit. By this experiment the rod free area can be determined directly. In the normal subject at 20′ in visual angle from the centre of the fovea, in the protanope at 5′ and in the detueranope at 10′ the rod process disappears. This result is in good agreement with our results obtained by the other method. It is, however, to be noted that in the present experiment we must always be careful to measure only the rod process, but not the blue process. If we

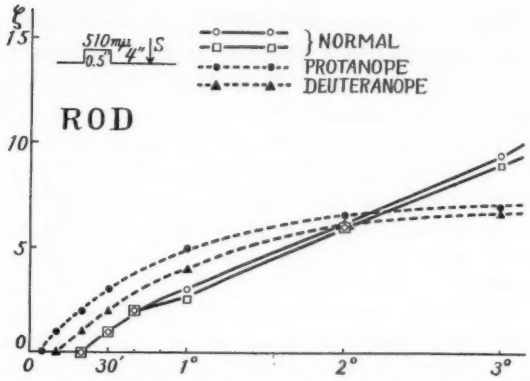

Fig. 4. Spatial distribution of rod process in fovea. Abscissas: Retinal position of test patch of 3′ in visual angle. Full curves refer to normal subjects. Broken curves to color-blind subjects.

are not certain that we are dealing with the rod process, then a ζ-time curve should be measured. In Fig. 5. the rod free area determined by our method, the histological data obtained by Polyak and by Oesterberg are shown schematically. As can be seen in this figure, our result for the normal subject is close to Oesterberg's result and is greatly different from Polyak's result.

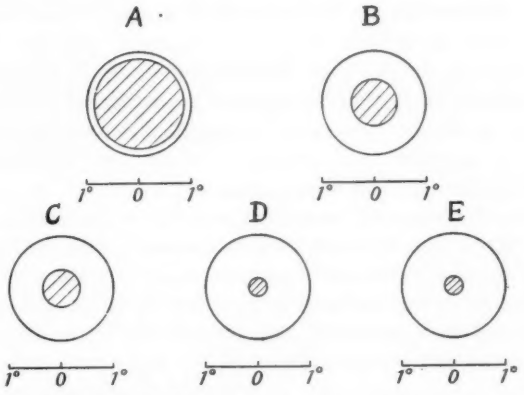

Fig. 5. Diagrams of rod free area.
A: Polyak's histological result.
B: Oesterberg's histological result.
C: Our result in normal subject.
D: Our result in protanope.
E: Our result in deuteranope.

Discussion

Oesterberg's and Polyak's histological findings with regard to the rod free area are different from each other. Nevertheless, various investigators have hitherto utilized either of these different findings, without careful scrutiny, in their researches on the function of the cones. According to Oesterberg's very careful observation, the rods appear first at 26.6′ from the center of the fovea in normal subject, in a area of 53.2′ in diameter in visual angle. In our experiments, the rods are found in areas greater than 50′ in diameter. So our result well agrees with Oesterberg's result.

In what follows the accuracy of our data will be discussed. In view of the fact that the duration of illumination was 0.5 seconds in all our experiments, the effect of physiological nystagmus has to be taken into account. According to Riggs,[17] under good experimental conditions the retinal image is virtually stationary for exposures up to 0.01 second. Exposures of 0.1 second entail an average displacement of 25″ of arc. Exposures as long as 0.5 seconds permit an average excursion of about 2′ of arc. Therefore, an average excursion of about 2′ of arc must be taken into consideration during 0.5 seconds exposure in our experiments. On account of this excursion of the retinal image, the rod free area obtained from Experiment 1 must be smaller by an amount of 2′ than the true rod free area. For the same reason the foveal position of each point in Experiment 2 becomes uncertain by the same amount $\pm 2'$.

Further we should take "stray light"[18] into consideration, because it is possible that stray light makes the measured rod free area smaller than it is. But the intensity of illumination used in Experiment 1 was so low especially in the neighborhood of the intensity range for the constant ζ that it is almost impossible to consider stray light as a source of error. This is, however, not altogether ruled out in Experiment 2 in which relatively strong light was used.

Moreover, the error of measurement of ζ values can have some influence upon the value of obtained rod free area. But this is of no concern in Experiment 1, because the intensity range for the constant ζ which determines the presence of the rods is hardly influenced by the error of measurement ± 2 in terms of ζ. In Experiment 2 the obtained size of the rod free area is directly influenced by such ζ-deviation. For that reason, in these experiments were used only well trained subjects in whom standard deviation of ζ was ± 0.3. This magnitude would correspond to $\pm 3'$ in diameter of the rod free area. In all respects Experiment 1 is superior to Experiment 2 for determination of the rod free area. As has been stated above there are two different types, type I and type II of ζ-intensity curves. The type II has been considered to be connected

with the photochromatic interval. If the rods have higher photosensitivity than the cones and if excitation of the rods gives a colorless sensation, the existence of the photochromatic interval may be considered to mean intervention of the rods. As some investigators, however, claim that excitation of the rods gives rise to a blue sensation and moreover as it is not so easy to measure the photochromatic interval, it would be difficult to determine the rod free area from psychophysical measurements of the photochromatic interval. So it is not adequate to consider the type II in relation to the existence of the photochromatic interval. Our results are, as a matter of fact, independent of this argument. In the type II which is characterized by the intensity range for the constant ζ the existence of the rod process has been proved experimentally. On the contrary, in the type I no rod process was shown to exist. So far as the rod process obtained by Motokawa's method is virtually a phenomenon based on rod excitation, it is right to investigate the rod free area by means of this method. That Motokawa's statement is right should be recognized in view of the fact that the spectral sensitivity curve determined by this method well agrees with the scotopic visibility curve.[12)]

The rod free areas of the color-blind subjects have been shown to be smaller than those of the normal subjects in our experiments. This fact is in line with the developmental aspects of color vision stated in Ladd-Franklin's theory. Because of insufficient differentiation the more primitive type of receptor, rods, must be distributed more widely in the fovea of the color-blind than in the fovea of the trichromat.

Summary

The distribution of the rod process was studied by the method of the electrical stimulation of the eye.

1) Experiment 1

The fovea of the human retina was exposed to circular patches of various sizes illuminated by spectral light of 510 mμ and ζ-intensity curves of the rod process were obtained. Two types of ζ-intensity curves were distingushed. In the type I ζ was a simple linear function of the logarithm of the intensity, and in the type II ζ remained constant over a certain range of lower intensities. It was shown that the latter behavior of type II is based on the rod process. Utilizing this property the rod free area was determined.

2) Experiment 2

The spatial distribution of the magnitude of ζ was obtained, using a patch of small size and thus the rod free area was directly determined.

3) The results of these experiments agreed with each other. Rods do not appear at all in a central area of 40′ in diameter in visual angle

and begin to appear in an area of 50′ in normal subjects. In color-blind subjects, protanope and deuteranope, rods do not appear at all in an area of 18′ and appear first in an area of 25′.

4) These results well agree with the rod free area determined histologically by Oesterberg.

The author wishes to express his hearty thanks to Prof. K. Motokawa for his kind guidance and encouragement.

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The Mechanism of Spatial Induction in the Retina

By

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Introduction

Spatial induction or simultaneous contrast is a familiar phenomenon. Therefore it is interesting to investigate how this phenomenon develops.

It has, however, been difficult to measure the course of development, based on sensations of contrast which are too vague to allow accurate measurements.

Motokawa[1] discovered a physiological correlate of simultaneous contrast and designated it " indirect induction " to be set against " direct induction " which is a certain physiological state found within a retinal area illuminated, and is a physiological correlate of successive contrast.

Indirect induction is a physiological state which can be found around a retinal area illuminated, and is in character complementary to direct induction.

In the present investigation, we studied the course of development of indirect induction and its propagation from the illuminated retinal area into the surrounding areas.

Experimental

Method

The method of electrostimulation consists in measuring the electrical excitability of the dark-adapted eye with a constant current of 0.1 sec., taking the least perceptible phosphene as an index, in order to measure an increase in electrical excitability after a conditioning photic stimulus.

When red, yellow, green and blue lights are used for conditioning, the electrical excitability reaches a maximum 1, 1.5, 2 and 3 sec. respectively after termination of the conditioning light.

If the white light is preceded by inducing colored light, the maximum of the electrical excitablity curve occurs at the time corresponding to the maximum of the curve for the colored light complementary to the inducing

light used. For example, the curve obtained by successive presentation of red and white resembles that for blue-green light, the crest time being 2.5 sec. for both curves. This is the phenomenon of direct induction.

If the subsequent white light is made to fall on a part adjacent to the retinal area pre-illuminated by red light, the excitability curve is obtained whose maximum lies at 1 sec., and it is obviously in character complementary to the curve obtained in the case of direct induction. This is the phenomenon of indirect induction. In this case, the red light, while acting on one part of the retina, induces a complementary or " blue-green " process in the adjacent area. Thus the process so induced plays the same role as blue-green inducing light in the experiment of successive induction. Accordingly the adjacent area tested by the white light yields an excitablity curve with a maximum at 1 sec. Similarly, when yellow, green and blue lights are used as inducing lights, the excitability curve obtained has a maximum at 1.5, 2 and 3 sec. respectively.

In all the experiment mentioned above, the excitability curve was measured at a long interval, e.g., 2 sec. between the inducing light and the white test-light.

It was shown by Motokawa[2] that when the duration of inducing light is sufficiently long, the magnitude of induction is independent of the interval between the inducing light and the subsequent white light. This is because induction fully develops during the inducing illumination. The question now arises as to how the magnitude of induction depends on the interval between the two lights when the inducing light is of shorter duration.

In Fig. 1, the dependence of excitability curves on the time interval between the two lights is illustrated.

The curve connecting open circles in this diagram represents the excitability curve for the white test-light alone, and the other curves refer to the data obtained with various time intervals between the red inducing light and the subsequent white light. The white light was made to fall on the adjacent area of the retinal part illuminated by the red inducing light. The duration of both illuminations was 40 msec. As can be seen in this diagram, the excitability curve becomes lower as the time interval between the two lights becomes shorter, but each curve has a maximum at 1 sec. When the interval is reduced to 60 msec., the curve obtained almost coincides with the curve for the white light alone. These examples indicate that the magnitude of induction, that is, the difference from the control curve increases as the interval between the two flashes is lengthened. In other words, some time has to be allowed for induction to develop in full extent. Thus, it has become clear that the time course of development of retinal induction can be studied by varying the time interval

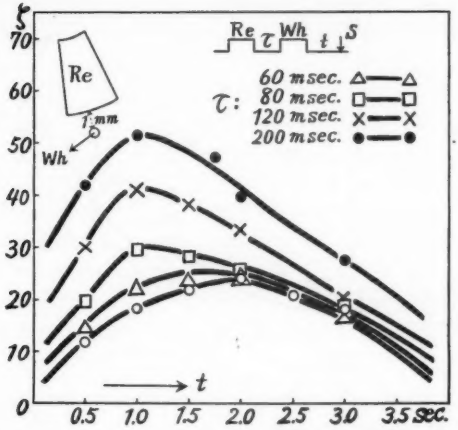

Fig. 1. Indirect induction caused by red light. Ordinates: percentage increases of electrical excitability above resting level. Abscissas: time in sec. after end of pre-illumination. The white test-patch was presented at a distance of 1 mm. from the margin of inducing patch. Exposure times for both patches were 40 msec.

between the two flashes of inducing and test lights.

As to the procedure for determination of electrical sensitivity the previous papers[3)4)] should be consulted.

The patches used for illumination consisted of a plate of ground glass covered by a sheet of black paper, from which two figures were cut, as are shown in Fig. 2, with patches drawn in broken lines I and T. The patch I is an inducing patch covered with a gelatin filter, and the T is a white test-patch 2 mm. in diameter. The gelatin filters used for the inducing patch were of the following characteristics. The range of transmission, tested spectroscopically with the light-adapted eye, was 600–650 mμ for the red filter, 530–630 mμ for the yellow filter, 500–600 mμ for the green filter and 430–500 mμ for the blue filter. These patches were presented in a vertical plane in front of the subject, the distance between the vertical plane and the eye being always 30 cm. The two patches were illuminated uniformly from behind with white light of 100 lux measured with a luxmeter. A pendulum of sector type, as shown in Fig. 2, was used in order to vary the interval between the two flashes.

SA in this diagram represents a shaft of the pendulum. SL_1 and SL_2 denote a fixed slit for exposure of the patch I and a movable slit for exposure of the patch T respectively. This pendulum was kept at an elevated position by means of an electromagnet, M. When released, the pendulum swung in a vertical plane at a distance of 28 cm. in front of

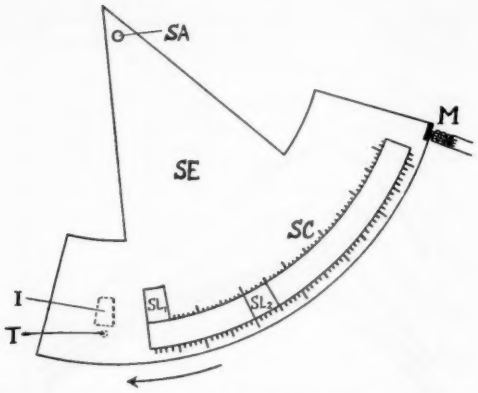

Fig. 2. Apparatus for successive presentation of inducing and white test patches at varying intervals.
SA: shaft. SE: sector. SC: scale. I: inducing patch. T: test patch. M: electromagnet. SL_1: fixed slit. SL_2: movable slit. Further explanation in text.

of eye of the subject.

By varying the distance between the two slits, the time interval between the two stimuli could be controlled. The subject had to catch the pendulum each time by his left hand lest it should swing back. Furthermore, after his response to an electric test stimulus, he had to bring the pendulum back into its elevated position. The interval between the two flashes and their duration were calibrated with a photocell and an oscillograph. Two examples of the calibration curves are shown in Fig. 3.

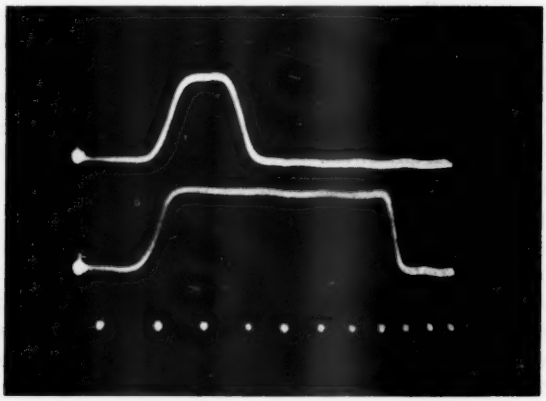

Fig. 3. Records used to calibrate exposure time. Time mark: 100 cps.

The fixation point of the eye was placed several mm. below the white test-patch. Other procedures will be described together with the results.

Results

Data are illustrated in Fig. 4, in which ordinates represent the contrast effects (C. E.), and abscissas the time interval between the two flashes. The contrast effect (C. E.) is defined as the difference between the maximal ordinate of the excitability curve obtained by the inducing procedure and the corresponding ordinate of the control curve for white light alone. The curves marked by crosses and by triangles in this diagram refer to the red and yellow inducing lights respectively. These curves indicate that indirect induction makes its appearance 20 msec. after the end of the inducing flash.

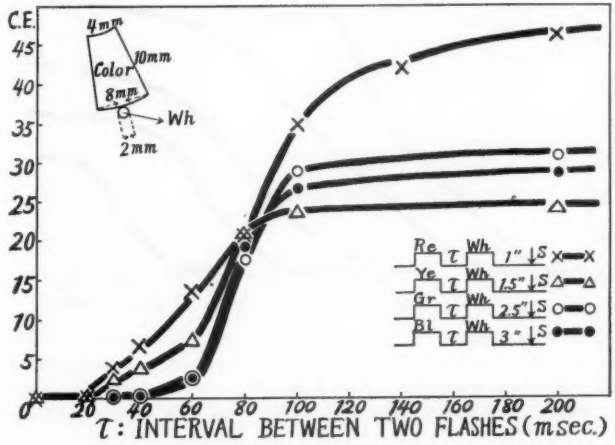

Fig. 4. Dependence of contrast effects on interval between two flashes, each lasting 40 msec. Arrangement of two patches and temporal sequence of procedure are shown in inset. Re, Ye, Gr, Bl, Wh and S denote red light, yellow light, green light, blue light, white light and a test electric stimulus respectively. The intensity of the white light was 100 lux.

When green or blue light is used as the inducing light, a greater delay is found; it starts 40 msec. after the end of the inducing flash. As the duration of colored light used was fixed at 40 msec., the latent period of induction caused by red or yellow light is 60 msec. when measured from the onset of the inducing stimulus, and that of induction caused by green or blue light 80 msec. It is, however, not clear whether the difference in latency depends on the wave-length of the inducing light, for the colored lights used were not of equal-energy. It is likely that the latency depends

not only on the wave-length, but also on the intensity of the inducing light.

In all the experiment mentioned above, indirect induction was measured in the immediately adjacent area of the retinal part illuminated by colored light. The following experiment was undertaken in order to measure the indirect induction established in a region remote from the retinal part illuminated by the colored light. As can be seen in inset of Fig. 5, the white test-patch was displaced step by step from the margin of the inducing patch. Red light was used as the inducing stimulus.

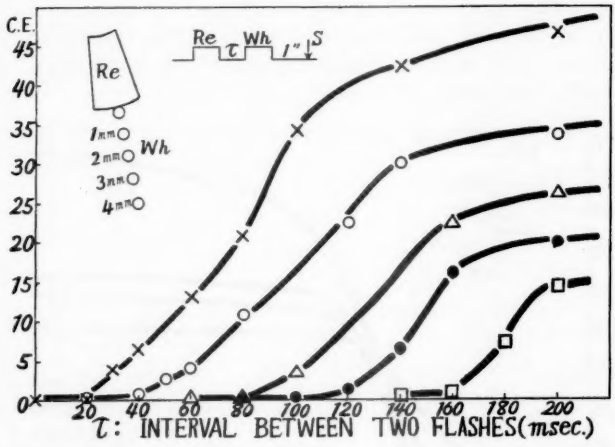

Fig. 5. Dependence of contrast effects on interval between two flashes. Red light (R), white light (Wh) and electric stimulus (S) were presented according to temporal sequence shown in inset. 5 curves from top downwards were obtained at a distance of 0, 1, 2, 3 and 4 mm. from margin of inducing patch.

In Fig. 5 data obtained at distances 0, 1, 2, 3 and 4 mm. from the margin of the inducing patch are represented by crosses, empty circles, triangles, solid circles and squares, respectively. As is shown in this diagram, the greater the distance from the inducing patch, the longer the latency of indirect induction. Moreover, indirect induction becomes weaker as the distance between the two patches increases. These facts may be explained as follows: 1) It takes certain time for indirect induction to be propagated from the retinal area illuminated by the colored light to the point at which indirect induction is measured. This accounts for the longer latencies with increasing distances. 2) The intensity of induction decreases as it is propagated, and this accounts for the decreasing magnitudes of induction with increasing distances. The

following quantitative treatment will strongly support this interpretation: Drawing a horizontal line of a definite C. E. value, say 5, and determining the point of intersection with each curve in Fig. 5, a series of time intervals are obtained at which the magnitude of induction at each measuring point attains the definite value. When the distances from the inducing patch to the measuring points are plotted against the time intervals so determined, a straight line is obtained, as is shown in Fig. 6. The straight lines marked by crosses and by triangles refer to the C. E. values 15 and 20, respectively. It is apparent that the slope of these straight lines represents the velocity of induction, and that the velocity is uniform throughout propagation. The fact that the three straight lines for C. E. values 5, 15 and 20 run parallel indicates that the same velocity is obtained whatever phase of propagation may be used for its determination.

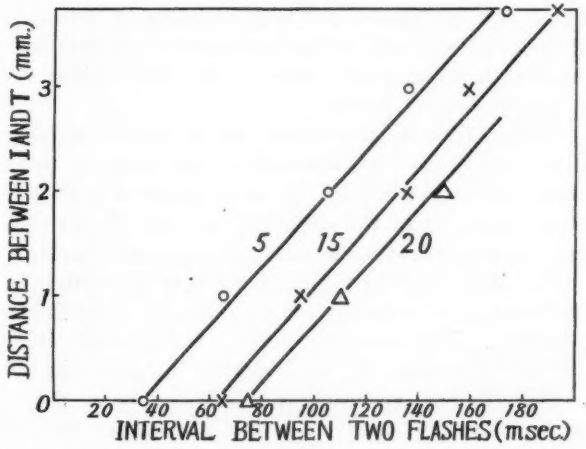

Fig. 6. Relation between time and distance traveled by spreading induction. Explanation about numbers 5, 15 and 20 in text.

The velocities of induction computed from the straight lines labeled 5 and 15 are found to be 29 and 31 mm./sec. respectively, 30 mm./sec. on the average.

When the posterior nodal distance of the eye is assumed to be 17 mm., a mean retinal velocity of 1.69 mm./sec. is obtained. It is to be noted that this value is in close agreement with that determined by Motokawa[5] with a quite different method of approach; he used "neutralization of retinal induction" for measurement of the velocity and obtained 1.7 mm./sec. on an average.

Discussion

As is shown in Fig. 5, the curves of indirect induction determined at various points increase hand in hand with the lapse of time after removal of the inducing light. Although not shown in this figure it takes about 300 msec. for the full development of indirect induction. According to Motokawa's experiment[1] retinal induction once developed remains unaltered for a period of about 20 sec., and then decreases gradually to disappear in 40 sec. or so. In this manner, retinal induction is characterized by its slow development and long persistence.

Of great importance is the fact that the velocity of propagation of induction measured above coincided with that measured by Motokawa based on a quite different principle. Motokawa found that the indirect induction caused by a colored light in a retinal area disappears on illuminating a remote retinal region by a light complementary to the former, and designated this phenomenon " neutralization of retinal induction." He further showed that such neutralization never occurs when there is an after-image of a white patch between the two regions of the retina illuminated by the colored lights.

Based on these facts he concluded that a process called " spreading induction " spreads to exert neutralization, and that the propagation of spreading induction is blocked by the after-image of a white patch.

In the measurement of the spreading velocity of this process Motokawa utilized the two phenomena, neutralization and blocking in a fascinating manner. Therefore the experiment is very interesting, in principle, but the experimental procedure is too complicated to be followed by any other investigators. The method used in the present experiment for measurement of the velocity is much simpler in principle as well as in procedure. Nevertheless, accuracy is as satisfactory as the complicated method by Motokawa. At any rate the satisfactory agreement of the data determined by these two different methods provides evidence for the unquestionable existence of spreading induction.

Summary

By the method of electrostimulation, the time course of development of indirect retinal induction was investigated. Indirect induction is a physiological correlate of simultaneous contrast.

1. Indirect induction appears with a latent period of 60 msec. in the immediate neighborhood of the retinal part illuminated by colored light.

2. The intensity of indirect induction increases with the lapse of time after removal of colored light, and reaches a constant level in about

300 msec.

3. At various distances from the area illuminated by colored light, development of indirect induction was measured, and it was found that the development is delayed in proportion to the distance. From this relation the velocity of spread of indirect induction was determined. The velocity was found to be 1.69 mm./sec. in the retina in complete agreement with the value determined by Motokawa with another method.

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Electrophoretic Study of Cerebrospinal Fluid Protein Components

Part II. Survey in Abnormal Liquor

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The object of the present study is analysing abnormal cerebrospinal fluid in the following three diseases; i.e., 1) schizophrenia, 2) epilepsy and 3) general paralysis.

With respect to the cerebrospinal fluid (liquor) protein components in schizophrenia, the total protein has been frequently discussed by many authors, among them Kafka,[1] Demme[2] and Guttmann[3] *et al.* reported a tendency to increase in slight degree, but on the contrary, Riebeling[4] and Hohmann[5] observed a tendency to decrease.

As for the liquor in epilepsy, the almost same problem has been discussed, but refrained from giving any final or definite conclusion.

There are a considerable number of reports on the liquor protein components in general paralysis, which definitely confirmed an increase of the total protein components. Particularly in reference to the total protein, an increase of the globulin fraction is regarded as its characteristic finding. Though rather dubious, there are various interpretations in reference to the relation between albumin and globulin as well as each fraction in globulin. There are a comparatively small number of literatures which were studied by means of the Tiselius electrophoretic apparatus, because this sort of research has been developed more recently. Consequently, the electrophoretic study on the pathological liquor protein components seems to be of great significance.

Methods and Subjects

In each case, the cerebrospinal fluid was obtained during the procedure of pneumoencephalography for the purpose of clinical diagnosis. The volume of the liquor varied between 80 and 200 m*l*. This liquor, part of which was examined by routine clincal methods,[6] was then experimentalized by the Kabat[7] and Fisk's[8] method which was recently

modified by the author.[9] It is of considerable importance that the author selected from a series of schizophrenics and epileptics such a liquor that satisfies the following factors: 1) no history of syphilis and alcohol-addiction, 2) negative Wassermann's reaction, including the one in the serum, 3) normal colloidal reaction and 4) normal pneumoencephalogram in particular. Before undertaking clinical treatment, the liquor puncture was previously made.

Results

Cerebrospinal fluid protein components in schizophrenia group

As shown in Table I, the total protein amount was estimated as high as 100 m*l*. in mean value, ranging from 18 to 40 mgm. The mobility of each fraction and the concentration of protein components are shown in Fig. 1 and Table II. Concerning the mobility of the liquor, it seems

TABLE I

Total Protein in mgm. per 100 m*l*.

Case No.	Age	Sex	Clinical diagnosis	Total protein mg%	Cell count
20	27	♂	Hebephrenia	22	3/3
21	43	♀	Hebephrenia	40	7/3
22	29	♀	Hebephrenia	30	1/3
23	26	♂	Hebephrenia	21	2/3
24	29	♂	Hebephrenia	18	0/3
27	34	♂	Hebephrenia	28	0/3
28	47	♂	Hebephrenia	35	4/3
45	23	♂	Hebephrenia	20	1/3
47	31	♀	Hebephrenia	18	4/3
51	24	♀	Hebephrenia	20	0/3
25	28	♀	Paranoid	22	3/3
26	29	♂	Paranoid	20	2/3
29	42	♂	Paranoid	33	6/3
30	27	♂	Katatonia	40	5/3
31	24	♀	Katatonia	23	1/3
		Mean		25.9	2.6/3

likely that the results correspond closely to the conditions recognizable in the normal liquor. Some cases showed to contain a small amount of the component designated as "X" which had such a mobility being faster than that of albumin, as shown in Fig. 1 (A).

Among those 15 schizophrenic cases studied, a clearly resolved com-

TABLE II

Concentration of Protein Components of Cerebrospinal Fluid in Schizophrenia

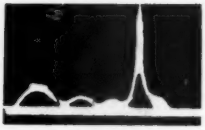
Case No.	Per cent composition					Mgm./100 ml. (calculated)				
	X	A	Globulin			X	A	Globulin		
			α	β	γ			α	β	γ
20	0.6	50.4	1.2	19.8	28.0	0.1	11.1	0.3	4.4	6.1
21		46.7	1.1	21.1	31.1		18.7	0.4	8.4	12.4
22	0.3	50.0	1.3	21.4	27.0	0.1	15.0	0.4	6.4	8.1
23	0.4	56.0	6.9	17.5	19.2	0.1	11.8	1.3	3.7	4.0
24		54.0	5.1	14.5	26.4		9.7	0.9	2.6	4.6
27		46.2	9.2	18.0	26.5		12.9	2.6	5.0	7.4
28	1.1	47.3	7.3	17.0	26.3	0.4	16.6	2.5	5.9	9.2
45	1.1	45.6	4.7	20.1	28.3	0.2	9.1	0.9	4.0	5.7
47		45.0	6.8	17.5	21.7		9.7	1.2	3.1	3.9
51	1.5	55.2	7.2	15.3	20.8	0.3	11.0	1.4	3.0	4.1
25		53.1	5.2	15.4	26.3		11.7	1.1	3.4	5.8
26	0.7	52.5	7.1	15.0	24.7	0.1	10.5	1.4	3.0	4.9
29	0.7	52.5	7.1	15.0	24.7	0.2	17.3	2.3	5.0	8.2
30	1.3	49.2	16.0	6.5	27.1	0.5	19.6	6.4	2.6	10.8
31	2.1	47.7	17.0	4.8	28.4	0.4	10.9	3.9	1.1	6.5
Mean	1.0	50.6	6.9	16.2	25.3	0.2	12.9	1.8	3.8	6.8

Where two values are given, the values refer to the composition of alpha-1 and alpha-2 or beta-1 and beta-2 components.

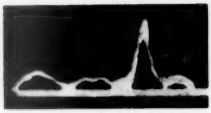
ponent with the anodic mobility being greater than albumin was recognized in 11 cases. The percent composition and the amount of each fraction in mg. per 100 m*l.* are also shown in Table II. Concerning the relation between albumin and globulin in the liquor protein in this group, it may be said that the results correspond closely to the conditions recognizable in the normal liquor.

Liquor protein components in epilepsy

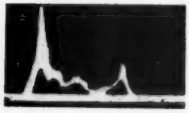
As shown in Table III, the mean value of the total protein amount was almost the same with the liquor in normal subject. The concentration of the protein components is shown in Table IV. The average value of the component designated as " X " which had such a mobility being faster than that of albumin, as shown in Fig. 1 and Table IV, was about 8.1% in the total spinal fluid proteins. The mean value of the X-component was 0.4 mg. per 100 m*l.* The relation between the globulin-per-albumin quotient and its relative values is shown in Table V.



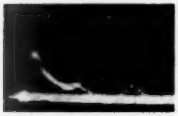
A. Schizophrenia



B. Epilepsy



C. General paralysis



D. General paralysis

Fig. 1. Electrophoretic patterns. (The descending limb only is shown.)

Mobility :

$$X=\frac{1.8\times\frac{64\times0.0082}{125}\times0.3}{3120\times0.008}=9.08\times10^{-5}\mathrm{Cm^2/Volt/Sec.}$$

$$\mathrm{Alb.}=\frac{1.6\times\frac{64\times0.0082}{125}\times0.3}{3120\times0.008}=8.07\times10^{-5}\mathrm{Cm^2/Volt/Sec.}$$

$$\alpha=\frac{1.2\times\frac{64\times0.0082}{125}\times0.3}{3120\times0.008}=6.06\times10^{-5}\mathrm{Cm^2/Volt/Sec.}$$

$$\beta=\frac{0.8\times\frac{64\times0.0082}{125}\times0.3}{3120\times0.008}=4.04\times10^{-5}\mathrm{Cm/Volt/Sec.}$$

$$\gamma=\frac{0.1\times\frac{64\times0.0082}{125}\times0.3}{3120\times0.008}=0.51\times10^{-5}\mathrm{Cm^2/Volt/Sec.}$$

Liquor protein findings in 11 *cases of general paralysis*

As shown in Table VI, the liquor protein findings in 11 cases of general paralysis were as follows: the mean value of the total protein amount was 158 mg. %; the mean value of the cell count, 55/3; Pandy, Nonne and Wassernann's reactions, positive. The per cent composition and the amount of each fraction in mg. per 100 m*l.* are also shown in VII. The mean value of gamma-globulin amount was 46.1% in the total protein amount. In 11 cases of general paralysis, a rapidly migrating X-component was not observed.

The relation between the globulin/albumin quotient and its relative value is shown in Table VIII.

The globulin/albumin ratio of the liquor in general paralysis was 2.9 in mean value.

TABLE III

Total Protein in mgm. per 100 m*l.* in Cerebrospinal Fluid in Epilepsy

Case No.	Age	Diagnosis present state	Total protein mg%	Cell count
82	16	Grand mal	18	7/3
83	38	Grand mal	20	7/3
84	14	Grand mal	24	2/3
85	11	Grand mal	42	5/3
86	14	Grand mal	20	1/3
87	24	Petit mal	30	0/3
88	9	Grand mal	20	2/3
89	21	Grand mal	10	8/3
90	13	Grand mal	18	2/3
91	26	Grand mal	20	4/3
92	28	Grand mal	21	2/3
93	31	Psychomotor	20	1/3
94	23	Psychomotor	18	0/3
		Mean	21.6	3/3

TABLE IV

Concentration of Protein Components of Cerebrospinal Fluid in Epilepsy

Case No.	Per cent composition					Mgm./100 m*l.* (calculated)				
	X	A	Globulin			X	A	Globulin		
			α	β	γ			α	β	γ
82	12	48	9	5	26	2.16	8.6	1.6	0.9	4.7
83	15	30	16	8	31	3.0	6.0	3.2	1.6	6.2
84	2	53	9	5	31	0.48	12.7	2.2	1.2	7.4
85		54	7	14.5	24.4		22.7	3.0	6.1	10.2
86	3	52	6	9	30	0.6	10.4	1.2	1.8	6.0
87	11	54	7	8	20	3.3	16.2	2.1	2.4	6.0
88		56	4	17	23		11.2	0.8	3.4	4.6
89		54	9	11	26		5.4	0.9	1.1	2.6
90	1.2	48	17	9	24.8	0.2	8.6	3.1	1.6	4.5
91	6	49	10	4	31	1.2	9.8	2.0	0.8	6.2
92	7	53	9	8	23	1.5	11.1	1.9	1.7	4.8
93	14	40	15	9	22	2.8	8.0	3.0	1.8	4.4
94	10	47	14	8	21	1.8	8.5	2.5	1.4	3.8
Mean	8.1	49	10.1	8.8	25.6	1.3	10.7	2.1	2.0	5.5

Where two values are given, the values refer to the composition of alpha-1 and alpha-2 or beta-1 and beta-2 components.

TABLE V

Relation between Albumin and Globulin in Cerebrospinal Fluid in Epilepsy

Case No.	Globulin / Albumin	Alpha-Glob. / Albumin %	Beta-Glob. / Albumin %	Gamma-Glob. / Albumin %	Albumin / Globulin	X-Frac. / Albumin %
82	0.83	18	10	54	1.2	25
83	1.83	53	26	103	0.5	50
84	0.84	16	9	58	1.2	3
85	0.85	13	26	45	1.2	
86	0.86	11	17	57	1.2	5
87	0.64	12	14	37	1.6	20
88	0.78	7	30	41	1.3	
89	0.85	16	20	48	1.2	
90	1.05	35	18	51	0.9	2
91	0.91	20	8	63	1.1	12
92	0.75	16	15	43	1.3	13
93	1.15	37	22	55	0.9	35
94	0.91	29	17	44	1.1	21
Mean	0.94	21.7	17.8	53.7	1.1	18.6

TABLE VI

Cerebrospinal Fluid Findings in 11 Case of General Paralysis

Case No.	Age	Present state	Total protein mgm.%	Cell count	Pandy reaction	Nonne reaction	Wasserman's reaction
71	51	Depressive form	116	55/3	###	###	###
72	50	Expansive form	110	42/3	###	###	###
73	27	Juvenile paralysis	170	38/3	###	###	###
74	33	Depreessive form	59	13/3	++	++	###
75	49	Dementia form	75	19/3	++	++	###
76	54	Expansive form	34	25/3	++	+	++
77	30	Dementia form	225	45/3	###	###	###
78	42	Depressive form	78	15/3	++	++	++
79	32	Dementia form	116	28/3	###	###	###
80	48	Expansive form	680	270/3	###	###	###
81	46	Depressive form	75	18/3	++	+	+
Mean			158	55/3			

TABLE VII

Concentration of Protein Components of Cerebrospinal Fluid in General Paralysis

Case No.	Per cent composition				Mgm./100 ml. (calculated)			
	A	Globulin			A	Globulin		
		α	β	γ		α	β	γ
71	22.6	3.2	17.7	56.5	26.2	3.7	20.5	65.5
72	11.0	14.0	23.0	52.0	12.1	15.4	25.3	57.2
73	23.8	4.2	23.5	48.2	40.4	7.1	39.9	81.9
74	41.0	2.6	10.3	46.1	24.1	1.5	6.0	27.2
75	25.5	5.5	22.6	47.4	19.1	4.1	16.9	35.5
76	25.0	5.0	20.4	49.6	8.5	1.7	6.9	16.8
77	24.6	5.6	20.8	49.0	55.3	12.6	46.8	110.0
78	41.0	3.1	21.7	34.2	31.9	2.4	16.9	26.6
79	25.4	3.7	25.5	45.4	29.4	4.2	29.5	52.6
80	35.0	2.5	13.3	49.2	238.0	17.0	90.4	334.5
81	49.0	3.6	17.8	29.6	36.7	2.7	13.3	22.2
Mean	29.4	4.8	19.7	46.1	47.4	6.6	28.4	75.5

Where two values are given, the values refer to the composition of alpha-1 and alpha-2 or beta-1 and beta-2 components.

TABLE VIII

Relation between Albumin and Globulin in the Cerebrospinal Fluid in General Paralysis

Case No.	Globulin / Albulin	Alpha-Glob. / Albumin %	Beta-Glob. / Albumin %	Gamma-Glob. / Albumin %	Albumin / Globulin
71	3.4	14.1	78.2	250	0.3
72	8.1	127.0	209.0	472	0.1
73	3.2	17.5	98.7	202	0.3
74	1.4	6.2	24.8	112	0.7
75	2.9	21.4	88.4	186	0.3
76	3.0	20.0	81.1	197	0.3
77	3.1	22.7	84.6	199	0.3
78	1.4	7.5	52.9	83	0.7
79	2.9	14.2	100.0	179	0.3
80	1.9	7.1	38.0	140	0.5
81	1.0	7.3	36.2	60	1.0
Mean	2.9	24.0	81.0	189	0.3

Comment

Almost all authors who had made investigations by former methods reported that an inconsiderable increase, above normal limits, of the total protein amount in the liquor was observed among schizophrenic psychosis and also considered that such changes would be due to an increase of albumin.[10] However, our results differed in some respects.

As shown in Table I, the tendency to increase was seen in 40% of the total number of patients and the tendency to decrease, in 13%. The mean value of the total protein amount was 25.9 mg. %, indicating the tendency to a slight increase than that of the total protein of the normal liquor. However, the fact that these average values are within normal limits should be emphasized.

The ratio of globulin/albumin was also ranged within the normal values. But, under the circumstance, the tendency to a slight increase was observed both in beta-globulin and gamma-globulin. (Table II).

X-component was also within the normal limits. This result was almost the same as that reviewed by Fisk and other workers.

If the liquor protein of schizophrenia were subjected to further discussion, the author suppose that the amino acid composed protein and the polysaccharid and lipoid combined with protein would be the main theme in the future.

With respect to the liquor protein of epilepsy, the total protein has been the subject in many previous literatures,[11),12)] in which the tendency to increase or to decrease seems to have been discussed mainly. According to the study by the author, the mean value of the total protein amount was almost the same as that in the normal liquor. (Table III). As shown in Table IV, it was observed that, in each fraction of the liquor protein, X-component, alpha-globulin and gamma-globulin increased relatively. This result should be differentiated from that of the studies by Fisk and other workers. As Hoch and Chanutin[13)] pointed out, an increase of " X-component " in the liquor protein of epilepsy was also recognized by the author. Fisk, Pieper[14)] and many other workers[15)16)17)] have studied about this X-fraction, but its substance was still uncertain. In view of the abundance of this X-component in the liquor protein of epileptic patients, it seems likely to be related somewhat with epileptic convulsion or seizures.

The characteristics of the liquor protein observable in general paralysis are an increase of the total protein amount and an increase of globulin involved in it. Consequently, the globulin/albumin ratio is usually above 1.0. In general paralysis, an increase of globulin in the liquor was previously considered to be due to an increase of gamma-globulin. This hypothesis would be verified again by the present study (s. Tables VI,

VII, VIII and Fig. 1).

Apart from our results, an approximately same value in the liquor protein in tabes dorsalis was also obtained by Ewerbeck.[18] Meanwhile, Rossi and Schneider[19] reported that, in case of "Hirnhautentzündung," albumin of the liquor protein decreased and gamma-globulin increased.

During the electrophoretic study on the liquor protein in 11 cases of general paralysis, X-component was observed in none of them. Gries and other workers[20] reported that, in case of an abnormal increase of the total protein in the liquor, X-fraction decreased and was, consequently, impossible to verify. We would like to agree with their conclusion, because our results showed certain resembling tendencies.

Summary

1. In this paper, the electrophoretic sutdy on the protein components of the cerebrospinal fluid in 15 cases of schizophrenia, 13 cases of epilepsy and 11 cases of general paralysis was reported.

2. Each fraction of liquor protein components in schizophrenia was almost the same as in normal liquor.

3. In case of epilepsy, the mean value of the toal protein amount was almost the same as that of the normal liquor, but X-component, alpha-globulin and gamma-globulin indicated a tendency to increase altogether. Particularly, X-component showed a remarkable increase.

4. None of X-component was observed in the liquor protein in cases of general paralysis, but gamm-globulin increased conspiculously and the globulin/albumin ratio was usually above 1.0.

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Errata : In the previous article described by the author entitled " Electrophoretic study of cerebrospinal fluid protein components, Part 1. Survey in normal liquor (this journal, Vol. 63, p. 245–250, 1956) ", the formulas in the page 248, consisting of X, Alb., Alpha, Beta and Gamma, should be corrected as follows: 0.08 to 0.008 and 10^{-6} to 10^{-5}.